DESCRIPTION

STAPHYLOCOCCUS AUREUS ANTIBACTERIAL TARGET GENES

RELATED APPLICATIONS

This application claims priority to Martin et al., *STAPHYLOCOCCUS AUREUS* ANTIBACTERIAL TARGET GENES, United States Provisional Application No. 60/003,798, filed September 15, 1995, and to Benton et al., *STAPHYLOCOCCUS AUREUS* ANTIBACTERIAL TARGET GENES, United States Provisional Application No. 60/009,102, filed December 22, 1995, which are incorporated herein by reference including drawings.

BACKGROUND

This invention relates to the field of antibacterial treatments and to targets for antibacterial agents. In particular, it relates to genes essential for survival of a bacterial strain *in vitro* or *in vivo*.

The following background information is not admitted to be prior art to the pending claims, but is provided only to aid the understanding of the reader.

Despite the development of numerous antibacterial agents, bacterial infections continue as a major, and currently increasing, medical problem. Prior to the 1980s, bacterial infections in developed countries could be readily treated with available antibiotics. However, during the 1980s and 1990s, antibiotic resistant bacterial strains emerged and have become a major therapeutic problem. There

are, in fact, strains resistant to essentially all of the commonly used antibacterial agents, which have been observed in the clinical setting, notably including strains of *Staphylococcus aureus*. The consequences of the increase in resistant strains include higher morbidity and mortality, longer patient hospitalization, and an increase in treatment costs. (B. Murray, 1994, *New Engl. J. Med.* 330:1229-1230.) Therefore, there is a pressing need for the development of new antibacterial agents which are not significantly affected by the existing bacterial resistance mechanisms.

Such development of new antibacterial agents can proceed by a variety of methods, but generally fall into at least two categories. The first is the traditional approach of screening for antibacterial agents without concern for the specific target.

The second approach involves the identification of new targets, and the subsequent screening of compounds to find antibacterial agents affecting those targets. Such screening can involve any of a variety of methods, including screening for inhibitors of the expression of a gene, or of the product of a gene, or of a pathway requiring that product. However, generally the actual target is a protein, the inhibition of which prevents the growth or pathogenesis of the bacterium. Such protein targets can be identified by identifying genes encoding proteins essential for bacterial growth.

SUMMARY

Each pathogenic bacterial species expresses a number of different genes which are essential for growth of the bacteria *in vitro* or *in vivo* in an infection, and which are useful targets for antibacterial agents. This invention provides an approach to the identification of those genes, and the use of those genes, and bacterial strains expressing mutant forms of those genes, in the identification, characterization, and evaluation of targets of antibacterial agents. It further provides the use of those genes and mutant strains in screening for antibacterial agents active against the genes, including against the corresponding products and pathways. Such active compounds can be developed into antibacterial agents. Thus, this invention also provides methods of treating bacterial infections in mammals by administering an antibacterial agent active against such a gene, and the pharmaceutical compositions effective for such treatment.

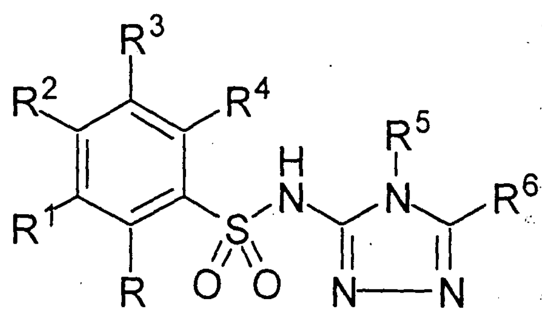
For the *Staphylococcus aureus* essential genes identified in this invention, the essential nature of the genes was determined by the isolation of growth conditional mutants of *Staphylococcus aureus*, in this case temperature sensitive mutants (ts mutants). Each gene was then identified by isolating recombinant bacteria derived from the growth conditional mutant strains, which would grow under non-permissive conditions but which were not revertants. These recombinant bacteria contained DNA inserts derived from the normal (*i.e.*, wild-type) *S. aureus* chromosome which encoded non-mutant products which replaced

the function of the products of the mutated genes. The fact that a clone having such a recombinant insert can complement the mutant gene product under non-permissive conditions implies that the insert contains essentially a complete gene, since it produces functional product.

The *Staphylococcal* genes described herein have either been completely sequenced or have been partially sequenced in a manner which essentially provides the complete gene by uniquely identifying the coding sequence in question, and providing sufficient guidance to obtain the complete sequence and equivalent clones. For example, in some cases, sequences have been provided which can be used to construct PCR primers for amplification of the gene from a genomic sequence or from a cloning vector, *e.g.*, a plasmid. The primers can be transcribed from DNA templates, or preferably synthesized by standard techniques. The PCR process using such primers provides specific amplification of the corresponding gene. Therefore, the complete gene sequence is obtainable by using the sequences provided.

In a first aspect, this invention provides a method of treating a bacterial infection in a mammal by administering a compound which is active against a bacterial gene selected from the group of genes corresponding to SEQ ID NO. 1-105. Each of these genes has been identified as an essential gene by the isolation of growth conditional mutant strains, and the complementation in recombinant strains of each of the mutated genes under non-permissive conditions, by expression from artificially-inserted DNA sequences

carrying genes identified by the specified sequences of SEQ ID NO. 1-105. In particular embodiments of this method, the infection involves a bacterial strain expressing a gene corresponding to one of the specified sequences, or a homologous gene. Such homologous genes provide equivalent biological function in other bacterial species. Also in a preferred embodiment, the compound has a structure described by the general structure below:



in which

R, R^1, R^2, and R^3 are independently H, alkyl (C_1-C_5), or halogen;

R^4 is H, alkyl (C_1-C_5), halogen, SH, or S-alkyl (C_1-C_3);

R^5 is H, alkyl (C^1-C^5), or aryl (C_6-C_{10});

R^6 is CH2NH2, alkyl (C1-C4), 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, or aryl (C_6-C_{10});

or

R^5 and R^6 together are $-C(R^7)=C(R^8)-C(R^9)=C(R^{10})-$, $-N=C(R^8)-C(R^9)=C(R^{10})-$, $-C(R^7)=N-C(R^9)=C(R^{10})-$, $-C(R^7)=C(R^8)-N=C(R^{10})-$, or $-C(R^7)=C(R^8)-C(R^9)=N-$;

in which

R^7, R^8, R^9, and R^{10} are independently H, alkyl (C_1-C_5), halogen, fluoroalkyl (C_1-C_5);

or

R^7 and R^8 together are -CH=CH-CH=CH-.

The term "alkyl" refers to a branched or unbranched aliphatic hydrocarbon group, e.g., methyl, ethyl, *n*-propyl, *iso*-propyl, and *tert*-butyl. Preferably the group includes from 1 to 5 carbon atoms and is unsubstituted, but alternativly may optionally be substituted with functional groups which are commonly attached to such chains, *e.g.*, hydroxyl, fluoro, chloro, aryl, nitro, amino, amido, and the like.

The term "halogen" refers to a substituent which is fluorine, chlorine, bromine, or iodine. Preferably the substituent is fluorine.

The term "pyridyl" refers to a group from pyridine, generally having the formula C_5H_4N, forming a heterocyclic ring, which may optionally be substituted with groups commonly attached to such rings.

The term furyl refers to a heterocyclic group, having the formula C_4H_3O, which may be either the alpha or beta isomer. The ring may optionally be substituted with groups commonly attached to such rings.

The term "thienyl refers to a group from thiophen, generally having a formula C_4H_3S

The term "aryl" refers to an aromatic hydrocarbon group which includes a ring structure in which the electrons are delocalized. Commonly, aryl groups contain a derivative

of the benzene ring. The ring may optionally be substitued with groups commonly attached to aromatic rings, e.g., OH, CH_3, and the like.

The term "fluoroalkyl" refers to an alkyl group, as described above, which one or more hydrogens are substituted with fluorine.

"Treating", in this context, refers to administering a pharmaceutical composition for prophylactic and/or therapeutic purposes. The term "prophylactic treatment" refers to treating a patient who is not yet infected, but who is susceptible to, or otherwise at risk, of a particular infection. The term "therapeutic treatment" refers to administering treatment to a patient already suffering from an infection

The term "bacterial infection" refers to the invasion of the host mammal by pathogenic bacteria. This includes the excessive growth of bacteria which are normally present in or on the body of a mammal. More generally, a bacterial infection can be any situation in which the presence of a bacterial population(s) is damaging to a host mammal. Thus, a mammal is "suffering" from a bacterial infection when excessive numbers of a bacterial population are present in or on a mammal's body, or when the effects of the presence of a bacterial population(s) is damaging the cells or other tissue of a mammal.

In the context of this disclosure, "bacterial gene" should be understood to refer to a unit of bacterial heredity as found in the chromosome of each bacterium. Each

gene is composed of a linear chain of deoxyribonucleotides which can be referred to by the sequence of nucleotides forming the chain. Thus, "sequence" is used to indicate both the ordered listing of the nucleotides which form the chain, and the chain, itself, which has that sequence of nucleotides. ("Sequence" is used in the same way in referring to RNA chains, linear chains made of ribonucleotides.) The gene includes regulatory and control sequences, sequences which can be transcribed into an RNA molecule, and may contain sequences with unknown function. The majority of the RNA transcription products are messenger RNAs (mRNAs), which include sequences which are translated into polypeptides and may include sequences which are not translated. It should be recognized that small differences in nucleotide sequence for the same gene can exist between different bacterial strains, or even within a particular bacterial strain, without altering the identity of the gene.

Thus, "expressed bacterial gene" means that, in a bacterial cell of interest, the gene is transcribed to form RNA molecules. For those genes which are transcribed into mRNAs, the mRNA is translated to form polypeptides. More generally, in this context, "expressed" means that a gene product is formed at the biological level which would normally have the relevant biological activity (*i.e.*, RNA or polypeptide level).

As used herein in referring to the relationship between a specified nucleotide sequence and a gene, the term "corresponds" or "corresponding" indicates that the

specified sequence identifies the gene. Therefore, a sequence which will uniquely hybridize with a gene from the relevant bacterium corresponds to that gene (and the converse). In general, for this invention, the specified sequences have the same sequence (a low level of sequencing error or individual variation does not matter) as portions of the gene or flanking sequences. Similarly, correspondence is shown by a transcriptional, or reverse transcriptional relationship. Many genes can be transcribed to form mRNA molecules. Therefore, there is a correspondence between the entire DNA sequence of the gene and the mRNA which is, or might be, transcribed from that gene; the correspondence is also present for the reverse relationship, the messenger RNA corresponds with the DNA of the gene. This correspondence is not limited to the relationship between the full sequence of the gene and the full sequence of the mRNA, rather it also exists between a portion or portions of the DNA sequence of the gene and a portion or portions of the RNA sequence of the mRNA. Specifically it should be noted that this correspondence is present between a portion or portions of an mRNA which is not normally translated into polypeptide and all or a portion of the DNA sequence of the gene.

Similarly, the DNA sequence of a gene or the RNA sequence of an mRNA "corresponds" to the polypeptide encoded by that gene and mRNA. This correspondence between the mRNA and the polypeptide is established through the translational relationship; the nucleotide sequence of the mRNA is

translated into the amino acid sequence of the polypeptide. Then, due to the transcription relationship between the DNA of the gene and the mRNA, there is a "correspondence" between the DNA and the polypeptide.

The term "administration" or "administering" refers to a method of giving a dosage of an antibacterial pharmaceutical composition to a mammal, where the method is, *e.g.*, topical, oral, intravenous, transdermal, intraperitoneal, or intramuscular. The preferred method of administration can vary depending on various factors, *e.g.*, the components of the pharmaceutical composition, the site of the potential or actual bacterial infection, the bacterium involved, and the severity of an actual bacterial infection.

The term "active against" in the context of compounds, agents, or compositions having antibacterial activity indicates that the compound exerts an effect on a particular bacterial target or targets which is deleterious to the *in vitro* and/or *in vivo* growth of a bacterium having that target or targets. In particular, a compound active against a bacterial gene exerts an action on a target which affects an expression product of that gene. This does not necessarily mean that the compound acts directly on the expression product of the gene, but instead indicates that the compound affects the expression product in a deleterious manner. Thus, the direct target of the compound may be, for example, at an upstream component which reduces transcription from the gene, resulting in a

lower level of expression. Likewise, the compound may affect the level of translation of a polypeptide expression product, or may act on a downstream component of a biochemical pathway in which the expression product of the gene has a major biological role. Consequently, such a compound can be said to be active against the bacterial gene, against the bacterial gene product, or against the related component either upstream or downstream of that gene or expression product. While the term "active against" encompasses a broad range of potential activities, it also implies some degree of specificity of target. Therefore, for example, a general protease is not "active against" a particular bacterial gene which produces a polypeptide product. In contrast, a compound which inhibits a particular enzyme is active against that enzyme and against the bacterial gene which codes for that enzyme.

The term "mammal" refers to any organism of the Class Mammalia of higher vertebrates that nourish their young with milk secreted by mammary glands, *e.g.*, mouse, rat, and, in particular, human, dog, and cat.

By "comprising" it is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or

mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

A DNA containing a specific bacterial gene is obtainable using a shorter, unique probe(s) with readily available molecular biology techniques. If the method for obtaining such gene is properly performed, it is virtually certain that a longer DNA sequence comprising the desired sequence (such as the full coding sequence or the full length gene sequence) will be obtained. Thus, "obtainable by" means that an isolation process will, with high probability (preferably at least 90%), produce a DNA sequence which includes the desired sequence. Thus, for example, a full coding sequence is obtainable by hybridizing the DNA of two PCR primers appropriately derived from the sequences of SEQ ID NO. 1-105 corresponding to a particular complementing clone to a *Staphylococcus aureus* chromosome, amplifying the sequence between the primers, and purifying the PCR products. The PCR products can then be used for sequencing the entire gene or for other manipulations. Those skilled in the art will understand the included steps,

techniques, and conditions for such processes. However, the full coding sequence or full gene is clearly not limited to a specific process by which the sequence is obtainable. Such a process is only one method of producing the final product.

A "coding sequence" or "coding region" refers to an open reading frame (ORF) which has a base sequence which is normally transcribed in a cell (*e.g.*, a bacterial cell) to form RNA, which in most cases is translated to form a polypeptide. For the genes for which the product is normally a polypeptide, the coding region is that portion which encodes the polypeptide, excluding the portions which encode control and regulatory sequences, such as stop codons and promoter sequences.

In a related aspect, the invention provides a method for treating a bacterial infection in a mammal by administering an amount of an antibacterial agent effective to reduce the infection. The antibacterial agent specifically inhibits a biochemical pathway requiring the expression product of a gene corresponding to one of the genes identified in the first aspect above. Inhibition of that pathway inhibits the growth of the bacteria *in vivo*. In particular embodiments, the antibacterial agent inhibits the expression product of one of the identified genes.

In the context of the coding sequences and genes of this invention, "homologous" refers to genes whose expression results in expression products which have a combination of amino acid sequence similarity (or base

sequence similarity for transcript products) and functional equivalence, and are therefore homologous genes. In general such genes also have a high level of DNA sequence similarity (*i.e.*, greater than 80% when such sequences are identified among members of the same genus, but lower when these similarities are noted across bacterial genera), but are not identical. Relationships across bacterial genera between homologous genes are more easily identified at the polypeptide (*i.e.*, the gene product) rather than the DNA level. The combination of functional equivalence and sequence similarity means that if one gene is useful, *e.g.*, as a target for an antibacterial agent, or for screening for such agents, then the homologous gene is likewise useful. In addition, identification of one such gene serves to identify a homologous gene through the same relationships as indicated above. Typically, such homologous genes are found in other bacterial species, especially, but not restricted to, closely related species. Due to the DNA sequence similarity, homologous genes are often identified by hybridizing with probes from the initially identified gene under hybridizing conditions which allow stable binding under appropriately stringent conditions (*e.g.*, conditions which allow stable binding with approximately 85% sequence identity). The equivalent function of the product is then verified using appropriate biological and/or biochemical assays.

In this context, the term "biochemical pathway" refers to a connected series of biochemical reactions

normally occurring in a cell, or more broadly a cellular event such as cellular division or DNA replication. Typically, the steps in such a biochemical pathway act in a coordinated fashion to produce a specific product or products or to produce some other particular biochemical action. Such a biochemical pathway requires the expression product of a gene if the absence of that expression product either directly or indirectly prevents the completion of one or more steps in that pathway, thereby preventing or significantly reducing the production of one or more normal products or effects of that pathway. Thus, an agent specifically inhibits such a biochemical pathway requiring the expression product of a particular gene if the presence of the agent stops or substantially reduces the completion of the series of steps in that pathway. Such an agent, may, but does not necessarily, act directly on the expression product of that particular gene.

The term "*in vivo*" in the context of a bacterial infection refers to the host infection environment, as distinguished, for example, from growth of the bacteria in an artificial culture medium (*e.g.*, *in vitro*).

The term "antibacterial agent" refers to both naturally occurring antibiotics produced by microorganisms to suppress the growth of other microorganisms, and agents synthesized or modified in the laboratory which have either bactericidal or bacteriostatic activity, *e.g.*, β-lactam antibacterial agents, glycopeptides, macrolides, quinolones, tetracyclines, and aminoglycosides. In general, if an

antibacterial agent is bacteriostatic, it means that the agent essentially stops bacterial cell growth (but does not kill the bacteria); if the agent is bacteriocidal, it means that the agent kills the bacterial cells (and may stop growth before killing the bacteria).

The term, "bacterial gene product" or "expression product" is used to refer to a polypeptide or RNA molecule which is encoded in a DNA sequence according to the usual transcription and translation rules, which is normally expressed by a bacterium. Thus, the term does not refer to the translation of a DNA sequence which is not normally translated in a bacterial cell. However, it should be understood that the term does include the translation product of a portion of a complete coding sequence and the translation product of a sequence which combines a sequence which is normally translated in bacterial cells translationally linked with another DNA sequence. The gene product can be derived from chromosomal or extrachromosomal DNA, or even produced in an *in vitro* reaction. Thus, as used herein, an "expression product" is a product with a relevant biological activity resulting from the transcription, and usually also translation, of a bacterial gene.

In another related aspect, the invention provides a method of inhibiting the growth of a pathogenic bacterium by contacting the bacterium with an antibacterial agent which specifically inhibits a biochemical pathway requiring the expression product of a gene selected from the group of

genes corresponding to SEQ ID NO. 1-105 or a homologous gene. Inhibition of that pathway inhibits growth of the bacterium. In particular embodiments, the antibacterial agent inhibits the expression product of one of the identified genes. Also in preferred embodiment, the antibacterial agent is a compound having a structure as described in the first aspect above.

The term "inhibiting the growth" indicates that the rate of increase in the numbers of a population of a particular bacterium is reduced. Thus, the term includes situations in which the bacterial population increases but at a reduced rate, as well as situations where the growth of the population is stopped, as well as situations where the numbers of the bacteria in the population are reduced or the population even eliminated.

A "pathogenic bacterium" includes any bacterium capable of infecting and damaging a mammalian host, and, in particular, includes *Staphylococcus aureus*. Thus, the term includes both virulent pathogens which, for example, can cause disease in a previously healthy host, and opportunistic pathogens which can only cause disease in a weakened or otherwise compromised host.

Similarly, the invention provides a method of prophylactic treatment of a mammal by administering a compound active against a gene selected from the group of genes corresponding to SEQ ID NO. 1-105 to a mammal at risk of a bacterial infection.

A mammal may be at risk of a bacterial infection, for example, if the mammal is more susceptible to infection or if the mammal is in an environment in which infection by one or more bacteria is more likely than in a normal setting. Therefore, such treatment can, for example, be appropriate for an immuno-compromised patient.

Also provided is a method of screening for an antibacterial agent by determining whether a test compound is active against one of the genes identified in the first aspect. In a particular embodiment the method is performed by providing a bacterial strain having a mutant form of a gene selected from the group of genes corresponding to SEQ. ID. NOS. 1-105 or a mutant gene homologous to one of those genes. The mutant form of the gene confers a growth conditional phenotype, *e.g.*, a temperature-sensitive phenotype, on the bacterial strain having that mutant form. A comparison bacterial strain having a normal form of the gene is also provided and the two strains of bacteria are separately contacted with a test compound under semi-permissive growth conditions. The growth of the two strains in the presence of the test compound is then compared; a reduction in the growth of the bacterial strain having the mutant form compared to the growth of the bacterial strain having the normal form of the gene indicates that the test compound is active against the particular gene.

In this context, a "mutant form" of a gene is a gene which has been altered, either naturally or

artificially, changing the base sequence of the gene, which results in a change in the amino acid sequence of an encoded polypeptide. The change in the base sequence may be of several different types, including changes of one or more bases for different bases, small deletions, and small insertions. By contrast, a normal form of a gene is a form commonly found in a natural population of a bacterial strain. Commonly a single form of a gene will predominate in natural populations. In general, such a gene is suitable as a normal form of a gene, however, other forms which provide similar functional characteristics may also be used as a normal gene. In particular, a normal form of a gene does not confer a growth conditional phenotype on the bacterial strain having that gene, while a mutant form of a gene suitable for use in these methods does provide such a growth conditional phenotype.

As used in this disclosure, the term "growth conditional phenotype" indicates that a bacterial strain having such a phenotype exhibits a significantly greater difference in growth rates in response to a change in one or more of the culture parameters than an otherwise similar strain not having a growth conditional phenotype. Typically, a growth conditional phenotype is described with respect to a single growth culture parameter, such as temperature. Thus, a temperature (or heat-sensitive) mutant (*i.e.*, a bacterial strain having a heat-sensitive phenotype) exhibits significantly reduced growth, and preferably no growth, under non-permissive temperature

conditions as compared to growth under permissive conditions. In addition, such mutants preferably also show intermediate growth rates at intermediate, or semi-permissive, temperatures. Similar responses also result from the appropriate growth changes for other types of growth conditional phenotypes.

Thus, "semi-permissive conditions" are conditions in which the relevant culture parameter for a particular growth conditional phenotype is intermediate between permissive conditions and non-permissive conditions. Consequently, in semi-permissive conditions the bacteria having a growth conditional phenotype will exhibit growth rates intermediate between those shown in permissive conditions and non-permissive conditions. In general, such intermediate growth rate is due to a mutant cellular component which is partially functional under semi-permissive conditions, essentially fully functional under permissive conditions, and is non-functional or has very low function under non-permissive conditions, where the level of function of that component is related to the growth rate of the bacteria.

The term "method of screening" means that the method is suitable, and is typically used, for testing for a particular property or effect in a large number of compounds. Therefore, the method requires only a small amount of time for each compound tested; typically more than one compound is tested simultaneously (as in a 96-well microtiter plate), and preferably significant portions of

the procedure can be automated. "Method of screening" also refers to determining a set of different properties or effects of one compound simultaneously.

Since the essential genes identified herein can be readily isolated and the gene products expressed by routine methods, the invention also provides the polypeptides encoded by those genes. Thus, the invention provides a method of screening for an antibacterial agent by determining the effects of a test compound on the amount or level of activity of a polypeptide gene product of one of the identified essential genes. The method involves contacting cells expressing such a polypeptide with a test compound, and determining whether the test compound alters the amount or level of activity of the expression product. The exact determination method will be expected to vary depending on the characteristics of the expression product. Such methods can include, for example, antibody binding methods, enzymatic activity determinations, and substrate analog binding assays.

It is quite common in identifying antibacterial agents, to assay for binding of a compound to a particular polypeptide where binding is an indication of a compound which is active to modulate the activity of the polypeptide. Thus, by identifying certain essential genes, this invention provides a method of screening for an antibacterial agent by contacting a polypeptide encoded by one of the identified essential genes, or a biologically active fragment of such a polypeptide, with a test compound,

and determining whether the test compound binds to the polypeptide or polypeptide fragment.

In addition, to simple binding determinations, the invention provides a method for identifying or evaluating an agent active on one of the identified essential genes. The method involves contacting a sample containing an expression product of one of the identified genes with the known or potential agent, and determining the amount or level of activity of the expression product in the sample.

In a further aspect, this invention provides a method of diagnosing the presence of a bacterial strain having one of the genes identified above, by probing with an oligonucleotide at least 15 nucleotides in length, which specifically hybridizes to a nucleotide sequence which is the same as or complementary to the sequence of one of the bacterial genes identified above. In some cases, it is practical to detect the presence of a particular bacterial strain by direct hybridization of a labeled oligonucleotide to the particular gene. In other cases, it is preferable to first amplify the gene or a portion of the gene before hybridizing labeled oligonucleotides to those amplified copies.

In a related aspect, this invention provides a method of diagnosing the presence of a bacterial strain by specifically detecting the presence of the transcriptional or translational product of the gene. Typically, a transcriptional (RNA) product is detected by hybridizing a labeled RNA or DNA probe to the transcript. Detection of a

specific translational (protein) product can be performed by a variety of different tests depending on the specific protein product. Examples would be binding of the product by specific labeled antibodies and, in some cases, detection of a specific reaction involving the protein product.

As used above and throughout this application, "hybridize" has its usual meaning from molecular biology. It refers to the formation of a base-paired interaction between nucleotide polymers. The presence of base pairing implies that at least an appreciable fraction of the nucleotides in each of two nucleotide sequences are complementary to the other according to the usual base pairing rules. The exact fraction of the nucleotides which must be complementary in order to obtain stable hybridization will vary with a number of factors, including nucleotide sequence, salt concentration of the solution, temperature, and pH.

The term, "DNA molecule", should be understood to refer to a linear polymer of deoxyribonucleotides, as well as to the linear polymer, base-paired with its complementary strand, forming double-strand DNA (dsDNA). The term is used as equivalent to "DNA chain" or "a DNA" or "DNA polymer" or "DNA sequence":, so this description of the term meaning applies to those terms also. The term does not necessarily imply that the specified "DNA molecule" is a discrete entity with no bonding with other entities. The specified DNA molecule may have H-bonding interactions with other DNA molecules, as well as a variety of interactions with other

molecules, including RNA molecules. In addition, the specified DNA molecule may be covalently linked in a longer DNA chain at one, or both ends. Any such DNA molecule can be identified in a variety of ways, including, by its particular nucleotide sequence, by its ability to base pair under stringent conditions with another DNA or RNA molecule having a specified sequence, or by a method of isolation which includes hybridization under stringent conditions with another DNA or RNA molecule having a specified sequence.

References to a "portion" of a DNA or RNA chain mean a linear chain which has a nucleotide sequence which is the same as a sequential subset of the sequence of the chain to which the portion refers. Such a subset may contain all of the sequence of the primary chain or may contain only a shorter sequence. The subset will contain at least 15 bases in a single strand.

However, by "same" is meant "substantially the same"; deletions, additions, or substitutions of specific nucleotides of the sequence, or a combination of these changes, which affect a small percentage of the full sequence will still leave the sequences substantially the same. Preferably this percentage of change will be less than 20%, more preferably less than 10%, and even more preferably less than 3%. "Same" is therefore distinguished from "identical"; for identical sequences there cannot be any difference in nucleotide sequences.

As used in reference to nucleotide sequences, "complementary" has its usual meaning from molecular

biology. Two nucleotide sequences or strands are complementary if they have sequences which would allow base pairing between the strands according to the usual pairing rules. This does not require that the strands would necessarily base pair at every nucleotide; two sequences can still be complementary with a low level of base mismatch such as that created by deletion, addition, or substitution of one or a few (up to 5 in a linear chain of 25 bases) nucleotides, or a combination of such changes.

Further, in another aspect, this invention provides a pharmaceutical composition appropriate for use in the methods of treating bacterial infections described above, containing a compound active on a bacterial gene selected from the group of genes described above and a pharmaceutically acceptable carrier. In a preferred embodiment, the compound has a structure as described in the first aspect above. Also, in a related aspect the invention provides a novel compound having antibacterial activity against one of the bacterial genes described above.

In a further related aspect a method of making an antibacterial agent is provided. The method involves screening for an agent active on one of the identified essential genes by providing a bacterial strain having a mutant form of one of the genes corresponding to SEQ ID NO. 1-105, or a homologous gene. As described above, the mutant form of the gene confers a growth conditional phenotype. A comparison bacterial strain is provided which has a normal form of said gene. The bacterial strains are contacted with

a test compound in semi-permissive growth conditions, and the growth of the strains are compared to identify an antibacterial agent. The identified agent is synthesized in an amount sufficient to provide the agent in a therapeutically effective amount to a patient.

A "carrier" or "excipient" is a compound or material used to facilitate administration of the compound, for example, to increase the solubility of the compound. Solid carriers include, *e.g.*, starch, lactose, dicalcium phosphate, sucrose, and kaolin. Liquid carriers include, *e.g.*, sterile water, saline, buffers, non-ionic surfactants, and edible oils such as peanut and sesame oils. In addition, various adjuvants such as are commonly used in the art may be included. These and other such compounds are described in the literature, *e.g.*, in the *Merck Index*, Merck & Company, Rahway, NJ. Considerations for the inclusion of various components in pharmaceutical compositions are described, *e.g.*, in Gilman et al. (Eds.) (1990); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press.

Consistent with the usage of "anti-bacterial agent" herein, the term "anti-bacterial activity" indicates that the presence of a particular compound in the growth environment of a bacterial population reduces the growth rate of that population, without being a broad cellular toxin for other categories of cells.

As is described below in the Detailed Description of the Preferred Embodiments, bacterial strains expressing a

mutated form of one of the above identified genes, which confers a growth conditional phenotype, are useful for evaluating and characterizing the gene as an antibacterial target and for screening for antibacterial agents. Therefore, this invention also provides a purified bacterial strain expressing a mutated gene which is a mutated form of one of the bacterial genes identified above, where the mutated gene confers a growth conditional phenotype.

Similarly, this invention provides a recombinant bacterial cell containing an artificially inserted DNA construct which contains a DNA sequence which is the same as or complementary to one of the above-identified bacterial genes or a portion of one of those genes. Such cells are useful, for example, as sources of probe sequences or for providing a complementation standard for use in screening methods.

The term "recombinant bacterial cell" has its usual molecular biological meaning. The term refers to a microbe into which has been inserted, through the actions of a person, a DNA sequence or construct which was not previously found in that cell, or which has been inserted at a different location within the cell, or at a different location in the chromosome of that cell. Such a term does not include natural genetic exchange, such as conjugation between naturally occurring organisms. Thus, for example, a recombinant bacterium could have a DNA sequence inserted which was obtained from a different bacterial species, or

may contain an inserted DNA sequence which is an altered form of a sequence normally found in that bacteria.

As described above, the presence of a specific bacterial strain can be identified using oligonucleotide probes. Therefore this invention also provides such oligonucleotide probes at least 15 nucleotides in length, which specifically hybridize to a nucleotide sequence which is the same as or complementary to a portion of one of the bacterial chains identified above.

In a related aspect this invention provides an isolated or purified DNA sequence at least 15 nucleotides in length, which has a nucleotide base sequence which is the same as or complementary to a portion of one of the above-identified bacterial genes. In particular embodiments, the DNA sequence is the same as or complementary to the base sequence of the entire coding region of one of the above-identified bacterial genes. Such an embodiment may in addition contain the control and regulatory sequence associated with the coding sequence.

Use of the term "isolated" indicates that a naturally occurring material or organism (*e.g.*, a DNA sequence) has been removed from its normal environment. Thus, an isolated DNA sequence has been removed from its usual cellular environment, and may, for example, be in a cell-free solution or placed in a different cellular environment. For a molecule, such as a DNA sequence, the term does not imply that the molecule (sequence) is the only molecule of that type present.

It is also advantageous for some purposes that an organism or molecule (*e.g.*, a nucleotide sequence) be in purified form. The term "purified" does not require absolute purity; instead, it indicates that the sequence, organism, or molecule is relatively purer than in the natural environment. Thus, the claimed DNA could not be obtained directly from total human DNA or from total human RNA. The claimed DNA sequences are not naturally occurring, but rather are obtained via manipulation of a partially purified naturally occurring substance (genomic DNA clones). The construction of a genomic library from chromosomal DNA involves the creation of vectors with genomic DNA inserts and pure individual clones carrying such vectors can be isolated from the library by clonal selection of the cells carrying the library.

In a further aspect, this invention provides an isolated or purified DNA sequence which is the same as or complementary to a bacterial gene homologous to one of the above-identified bacterial genes where the function of the expression product of the homologous gene is the same as the function of the product of one of the above-identified genes. In general, such a homologous gene will have a high level of nucleotide sequence similarity and, in addition, a protein product of homologous gene will have a significant level of amino acid sequence similarity. However, in addition, the product of the homologous gene has the same biological function as the product of the corresponding gene identified above.

Similarly, the invention provides an isolated or purified DNA sequence which has a base sequence which is the same as the base sequence of a mutated bacterial gene selected from one of the genes identified in the first aspect where the expression of this DNA sequence or the mutated bacterial gene confers a growth conditional phenotype in the absence of expression of a gene which complements that mutation. Such an isolated or purified DNA sequence can have the base sequence which varies slightly from the base sequence of the original mutated gene but must contain a base sequence change or changes which are functionally equivalent to the base sequence change or changes in the mutated gene. In most cases, this will mean that the DNA sequence has the identical bases at the site of the mutation as the mutated gene.

As indicated above, by providing the identified essential genes, the encoded expression products are also provided. Thus, another aspect concerns a purified, enriched, or isolated polypeptide, which is encoded by one of the identified essential genes. Such a polypeptide may include the entire gene product or only a portion or fragment of the encoded product. Such fragments are preferably biologically active fragments which retain one or more of the relevant biological activities of the full size gene product.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the fold increase in sensitivity toward 12 antibacterial agents and a generally toxic agent for 3 temperature sensitive mutants of *Salmonella typhimurium*. These are mutants of DNA gyrase subunit A (*gyrA212, gyrA215, and gyrA216*, grown at a semi-permissive temperature (35_C). Hypersensitivity is observed to antibacterial agents acting on DNA gyrase, but not to other classes of drugs or toxic agents. The data demonstrate that growth conditional mutations in a known target cause hypersensitivity to target inhibitors.

Fig. 2 presents the hypersensitivity profiles of a set of temperature sensitive mutants of *Salmonella*, for a variety of antibacterial agents with characterized modes of action, compared to the sensitivity profile of wild type.

Fig. 3 illustrates a variety of types of interactions which exist between different essential genes, and which can create differential responses in screens using growth conditional mutants.

Fig. 4 illustrates a possible arrangement of a multichannel screen plate using conditional growth mutants with mutations affecting 5 different cellular processes plus controls.

Fig. 5 illustrates 2 alternative multichannel screen designs in which either multiple compounds are screened using a single growth conditional mutant on each plate, or in which multiple growth conditional mutants are

used on each plate to create an inhibition profile of a single compound.

Fig. 6 is a bar graph showing the different heat sensitivity proviles for 6 *S. aureus* heat sensitive mutant strains. The growth of each strain is shown at 6 different temperatures ranging from 30°C to 43°C.

Fig. 7 is a bar graph showing the different heat sensitivity profiles for 4 different *S. aureus* polC heat sensitive mutants and a wild type strain. The growth of each strain is shown at 6 different temperatures ranging from 30°C to 43°C.

Fig. 8 is a graph showing the differences in hypersensitivity of one *S. aureus* heat sensitive strain (NT99) toward 30 inhibitory compounds at 3 different temperatures.

Fig. 9 is a diagram for two *S. aureus* mutants, illustrating that a greater number of growth inhibitory hits are identified at higher temperatures using heat sensitive mutants. Compounds were identified as hits if the growth of the mutant was inhibited by at least 50% and the inhibition of growth of the mutant was at least 30% higher than the inhibition of growth of a wild type strain.

Fig. 10 is a bar diagram illustrating the effect of test compound concentration on the number of hits identified, showing that, in general, more compounds are identified as hits at higher concentrations.

Fig. 11 presents the structures of two compounds which exhibited the same inhibition profiles for a set of

temperature sensitive *Staphylococcus aureus* mutants, showing the structural similarity of the compounds.

Fig. 12 presents the fold increase in sensitivity of a set of *Staphylococcus aureus* temperature sensitive mutants for a variety of compounds which inhibit growth of *Staphylococcus aureus* wild type, but which have uncharacterized targets of action.

Fig. 13 illustrates the types of anticipated inhibition profiles of different growth conditional mutants for a variety of test compounds, indicating that the number of mutants affected by a particular compound is expected to vary.

Fig. 14 shows the proportion of compounds (from a total of 65) which significantly inhibited the growth of varying numbers of temperature sensitive mutants in a screen of uncharacterized growth inhibitors of *Staphylococcus aureus*.

Fig. 15 shows the potency (MIC values) of a number of growth inhibitors which affected 0, 1 or more than 3 temperature sensitive mutants of *Staphylococcus aureus* in a screen of uncharacterized growth inhibitors.

Fig. 16 shows the number of hits for each of the temperature sensitive mutants of *Staphylococcus aureus* in a screen of 65 uncharacterized growth inhibitors.

Fig. 17 shows some advantages of a multichannel genetic potentiation screen using growth conditional mutants over traditional biochemical screens with either a known target or an unknown cloned gene.

Fig. 18 illustrates a strategy for selecting dominant lethal mutants for use in screens for antibacterial agents, not requiring hypersensitivity.

Fig. 19A-D are structures of four compounds which were identified as hits on mutant NT94.

Fig. 20 is a partial restriction map of the *S. aureus* clone insert (complementing mutant NT64), showing the position of the initial left and right sequences obtained.

Figs. 21-90 are partial restriction maps of each of the *S. aureus* clone inserts for which sequences are described herein, showing the relative fraction of the insert for which nucleotide sequence is described, as well as the approximate positions of identified open reading frames (ORFs).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. General Approach for Identification of Target Genes

As was briefly described in the Summary above, this invention concerns essential genes in *Staphylococcus aureus*. This organism is a serious pathogen which frequently carries resistance to a variety of existing antibiotic agents. Such resistant strains of *S. aureus* are a particular problem in settings where antibacterial agents are intensively used, such as in hospitals. To overcome the therapeutic difficulties posed by the existing resistant strains, it is highly desirable that new classes of antibiotic drugs be found, particularly ones which are active against new bacterial targets. While such bacterial

targets are usually (though not always) proteins, the targets can be identified by first identifying the bacterial genes which encode proteins (or RNA transcripts) that are essential for growth of the bacteria.

Identification of these genes which are essential for growth of the bacteria was accomplished by isolating conditional lethal mutant strains. Such mutant strains will grow under permissive conditions, but will not grow, or grow very poorly under non-permissive conditions. For the bacterial genes described herein, temperature sensitive mutants provided the growth conditional phenotype. The particular gene in each strain which was mutated to confer a growth conditional phenotype was then identified by isolating recombinant derivatives of the mutant strains. These recombinant strains each contained a DNA insert which, when expressed, would complement the defective gene and thus would allow growth under non-permissive conditions. These DNA inserts were provided by a genomic library of a normal *S. aureus* chromosome. The ability of the DNA insert in the recombinant strain to complement the defective product of the mutated gene showed that the DNA insert contained essentially a complete gene corresponding to a particular mutated gene. The vectors carrying each of these DNA inserts were constructed such that the *S. aureus* chromosomal insert could be amplified by PCR using flanking primer sequences. Each of the amplified *S. aureus* inserts was then partially sequenced, in general from both the 5' and 3' ends. This sequencing was, in general, single pass

sequencing and, thus, the specified sequences may contain a low level of sequence errors compared to the actual gene sequence. Since the partial sequences at the 5' and 3' ends bracket the complete gene, such partial sequences uniquely identify and provide that complete gene without interference from a low level of sequencing error. The complete gene and gene sequence can be reliably obtained by any of several different methods. For example, probes can be constructed based on the partial sequences provided, which can be used to probe genomic or cDNA libraries of *S. aureus*. Clones containing the corresponding 5' and 3' sequences can then be further characterized and sequenced to provide the complete gene. In another approach, the partial 5' and 3' sequences can be used to construct PCR primer sequences which can be used to amplify the sequence between those primers and likewise provide the complete gene. In yet another approach, equivalent growth conditional mutant strains can be obtained by following the same or a similar process of mutagenizing the base *S. aureus* strain, and then likewise obtaining the complete gene by isolating complementing clones which correspond to the sequences provided, from a genomic or cDNA library. It should again be noted that, for any of these approaches, a low level of sequencing error in the sequence presented herein does not matter, since the stringency of the hybridizing conditions can be readily adjusted to provide the appropriately specific binding.

While the genes identified in this invention are highly useful as targets for novel antibacterial therapy, the genes

and parts of those genes are also useful to provide probes which can be used to identify the presence of a particular bacteria carrying a particular gene. In addition, the growth conditional mutant strains described above are also useful as tools in methods for screening for antibacterial agents which target that gene (targeting the corresponding normal gene). The methods involved in the identification of the mutant strains complementing recombinant clones and the particular genes are described in more detail below.

A. Bacterial strain selection

The growth conditional mutant strains and recombinant strains herein are based on *S. aureus* strain 8325-4. This strain has been the subject of substantial genetic characterization and is appropriate for use in the approach described herein. It is believed to be free of transposons, phage or extrachromosomal elements. Numerous other strains of *S. aureus* can likewise be used. However, it is advantageous to select a strain which has few, or preferably no, transposons or extrachromosomal elements, as such elements can complicate the genetic analysis.

B. Isolation of conditional lethal mutants (general).

Heat-sensitive mutants were obtained after diethyl sulfate (DES; SIGMA Chemical) mutagenesis of strain 8325-4. Briefly, single colonies were inoculated into LB broth in individual wells of a 96-well microtiter plate and grown overnight (35°C, 18 h). Culture supernatants (10 μl) were diluted into λ-dilution buffer (λdil; 500 μl) and then treated with DES (5 μl). After a short incubation period

(20 min at 37°C), the treated cultures were serially diluted with λdil into microtiter plates. After an additional incubation period (8-12 h. at 37°C), appropriate dilutions (50 μl each of 10 E-2 and 10 E-3) were plated onto TS agar plates and incubated overnight (30°C, 18 h). The plates were replica-printed onto two Tryptic-soy (TS) plates and incubated either at 30°C or 43°C (permissive and non-permissive conditions, respectively). Colonies growing at 30°C but not at 43°C were isolated and their ts phenotype was subsequently confirmed in a second round of plating. Only one ts mutant was picked from an original singe-colony culture to assure that the mutants isolated were independent from each other. Independently-derived colonies with the appropriate phenotype are identified by direct screening on rich solid media at a permissive temperature (30°C), as it obviates selection of mutants deficient in metabolic pathways, such as aromatic amino acid biosynthesis. No penicillin enrichment is employed, as it would counterselect mutant strains that are strongly bactericidal at the non--permissive temperature. A preliminary collection of 100 independent condition-lethal mutants and 71 non-independent mutants was made. This collection has been supplemented with additional condition-lethal mutants.

C. Creation of the *S. aureus* shuttle library

The *S. aureus* strain used for the preparation of genomic DNA for library construction as well as for the generation of conditional-lethal (temperature sensitive) mutants described in this document is a derivative of NCTC

8325, designated as 8325-4 (Novick, R.P., 1990). The 8325 parent strain is one of the better-characterized strains of *S. aureus*, with genetic and physical map data available in the current literature (Pattee, P.A., 1990). The 8325-4 derivative strain has all the chromosomal elements of the parent, with the exception of integrated (*i.e.*, prophage and transposon DNA) and extrachromosomal (*i.e.*, plasmid DNA) elements endogenous to the parent.

Cloning and subcloning experiments utilized the commercially-available *E. coli* strains JM109 (Promega) and DH5alpha (GIBCO-BRL). All enzymes cited (*i.e.*, restriction endonucleases, ligases and phosphatases) were obtained commercially (NEB, Promega). All DNA cloning and manipulations are described in the current literature (Sambrook, et al., 1989). Parent plasmids pE194 and pUC19 have been described previously (Horinouchi, S. et al., 1982; Yanisch-Perron, C. et al., 1985) Recombinant constructs for use in a *S. aureus* host were first electroporated (Gene Pulser, BioRad) into *S. aureus* strain RN4220 (a restriction-deficient but methylase-proficient strain; Novick, R.P., 1990) before transduction into the target strain for complementation and cross-complementation analyses.

D. Library Construction

The shuttle plasmid vector used was pMP16, constructed by cloning the entire length of the natural *S. aureus* plasmid pE194 (linearized with Cla I) into the Nar I site of pUC19 (Yanisch-Perron et al., 1985). This new construct replicates and offers antibiotic resistance

selections in both *E. coli* and *S. aureus*. It also provides blue-white screening to facilitate scoring of insert-containing clones. Carefully purified genomic DNA from *S. aureus* strain 8325-4 was partially digested (Sau3A I) and fragments of 2-8 kb were isolated by sucrose gradient centrifugation. DNA fragments isolated in this manner were then used for constructing two different libraries. In library A, the DNA fragments were directly cloned into pMP16, which had been linearized (Bam HI) and dephosphorylated (CIP). The DNA mixture was ligated (T4 DNA ligase) and transformed into *E. coli* DH5alpha. Library A thus constructed contains about 60,000 independent clones, 60% of which have inserts. In constructing library B, the ends of the Sau3A I fragments were partially filled with dGTP and dATP, ligated with linearized (Sal I) pMP16 that was partially filled with dCTP and dTTP, and transformed into *E. coli*. The advantage of partially filling the ends is that DNAs with the same ends can no longer ligate to each other; the majority of the ligation occurs between the vector and inserts, significantly increasing the percentage of insert-containing clones. In addition, the chance that two unrelated insert fragment are fortuitously ligated in the same clone is greatly reduced by using this strategy. Library B consists of 50,000 independent clones with > 98% containing inserts. Both library A and library B contain at least a 50-fold representation of the *S. aureus* genome.

Clones from the two libraries were pooled and plasmid DNA extracted. The DNAs were used to transform *S.*

aureus strain RN4220. About 100,000 erythromycin resistant transformants were pooled and infected with bacteriophage ϕ11 at a multiplicity of infection (MOI) of 0.01 to generate phage lysates containing the shuttle library plasmids. The lysates were then used to introduce the shuttle plasmids into ts mutants by transduction to isolate complementing clones.

E. Isolation of complementing clones (general)

The lysate from library B was first chosen for transduction of the ts mutants because of its higher insert frequency. The ts mutants were grown either in TS broth or on TS agar plates overnight (18 h). The cells were resuspended in TS broth containing $CaCl_2$ (5 mM) to an OD_{600} between 2 - 3. The lysate from library B (10-50 μl) was added to the resuspended cells (2 ml) and incubated at 30°C with slow shaking (20 m). Ice-cold sodium citrate (20 mM; 1 ml) was added and the culture was centrifuged to pellet the cells. After removing the supernatant, the pellet was resuspended in ice-cold sodium citrate (20 mM; 500 μl). A small aliquot (about 1/5000 of the total volume) was plated on a TSA-ery-citrate plate (TS agar containing 5 μg/ml erythromycin and 500 μg/ml sodium citrate) and incubated at 30°C overnight (18 h). The total number of erythromycin-resistant transductants screened were estimated from this plate; at least 200,000 transductants were screened for each ts mutant to assure that the library population was well represented. The rest of the cells were plated onto the same selection media (3-5 plates), incubated

at 30°C for 5 h and then at 43°C overnight (18 h). Individual colonies that appeared on the 43°C plates were isolated and infected with ϕ11 to generate lysates.

The lysates prepared from these individual colonies were then used to transduce the same ts mutants as described above, using much smaller volumes of cells (0.1 ml) and lysates (1-3 μl) to facilitate testing of large number of lysates. Equal amounts of the transduced cultures were plated onto two sets of TSA-ery-citrate plates and incubated at either 30 or 43°C. Individual lysates that generated similar numbers of transductants at 30 and 43°C were scored as complementing clones. Among the first 96 ts mutants studied, complementing clones were isolated for 60 (63%) of the mutants; 57 were from library B and 3 were from library A.

To test whether different ts mutants carry mutations in the same or closely linked genes, cross complementation was performed to evaluate the ability of positive clones of one ts mutant to complement another mutant. The results showed that, while some positive clones failed to complement any ts mutants other than their primary mutant, other clones were able to complement additional mutants. Taken together, the cross complementation studies identified 38 loci on the *S. aureus* chromosome, each consisting of at least one essential gene.

All the positive clones for the 60 ts mutants were twice streaked on TSA-ery-citrate plates and grown at 43°C to eliminate ϕ11 prophage from the host cells. Plasmid DNA

was extracted from these complementing clones and transformed into *E. coli*. The plasmids were prepared from the *E. coli* clones and used for restriction mapping and subcloning of the inserts.

F. Strategy for DNA sequencing of complementing clones (general)

Complementing clones were subcloned into a sequencing vector (pGEM3Zf(+); Promega) containing regions of DNA flanking the multiple cloning site (T7 and SP6 primer annealing sites) to facilitate plasmid-based automated sequencing. Clones larger than 1.54 kB were cut with restriction endonucleases (BamHI, HindIII, EcoRI; NEB) and then subcloned into the same sequencing vector. DNA sequence ladders were generated by thermocycle sequencing procedures based upon the use of fluorescent-labeled primers (one of T7, SP6, M13 forward and M13 reverse; ABI), a thermostable DNA polymerase (AmpliTaq; Perkin Elmer/ABI) and dideoxy terminator chemistry (Sanger, et al, 1977, *Proc. Natl. Acad. Sci. USA* 74:54463). Data were acquired on an ABI 373A automated DNA sequencer (ABI) and processed using the PRISM sequence analysis software (ABI). The nucleotide sequences disclosed herein represent the range of highest quality data acquired in one pass for each clone. All DNA sequence data are reported with the same directionality, 5′ to 3′, regardless of which strand (i.e., coding or anti-coding) is sequenced. Some DNA sequence is reported using standard IUB codes in cases where sequence ambiguities could not be absolutely resolved in first-pass sequence.

For the sequences identified herein as SEQ ID NO. 1-105, the sequences corresponding to each complementing clone identify and provide the coding sequence (gene) responsible for providing that complementation. Therefore, the sequences corresponding to each complementing clone correspond to a particular essential gene.

G. DNA sequence analysis of complementing clones

Similarity searching (general)

Sequence data were analyzed for similarity to existing publicly-available database entries both at the nucleic acid level and the (putative) polypeptide level; the current releases and daily cumulative updates of these databases are maintained at the NCBI and are freely accessible. The programs BLASTN (Altschul, et al., 1990, *J. Mol. Biol.* 215:403-410) and FASTA (Pearson, et al., 1988, *Proc. natl. Acad. Sci. USA* 85:2444-2448) were used to search the nucleic acid databases GenBank (Release 89.0) and EMBL (Rel. 43.0), while the programs BLASTX and TFASTA were used to search the protein databases SwissProt (Rel. 30.0), PIR (Rel. 45.0) and GenPept (Rel 89.0). For reporting the results of the similarity searching below, the following abbreviations of bacterial species names are used:

Bsu = Bacillus subtilis
Eco = Escherichia coli
Zmo = Zymomonas mobilis
Bme = Bacillus megaterium
Lme = Leuconostoc mesenteriodes
Sxy = Staph. xylosys
Sca = Staph. carnosus
Sau = Staph. aureus
Hin = Haemophilus influenzae
Seq = Strep. equisimilis

Bca = Bacillus caldolyticus
Kpn = Klebsiella pneumoniae
Mle = Mycobacterium leprae

H. DNA Sequence of Complementing Clones

Mutant NT 6 - Clone pMP33: an example of complementing ORFs with literature precedent in *Staph. aureus*.

The ORF complementing the heat-sensitive phenotype of *S. aureus* mutant *NT6* described here was identified by sequencing subclones of pMP33, an *E. coli/S. aureus* shuttle vector containing a 2.3 kilobase-pair (kb) insert of parental (i.e. wild-type) genomic DNA. The subclones, pMP1006 (0.5kb), pMP1007 (0.9 kb) and pMP 1008 (0.9 kb), were generated by EcoRI and HindIII digestion of the parent clone and ligation into pGEM3Zf(+), a commercially available vector (Promega, Inc.) suitable for double-stranded DNA sequencing applications.

PCR-based methods (PRISM Dye Primer DNA Sequencing Kit; ABI, Inc.) were employed to generate DNA sequence data from the SP6 promoter of each of the subclones. Electrophoresis and detection of fluorescently-labelled DNA sequence ladder on an ABI 373A automated DNA sequencer (ABI, Inc.) yielded the following sequence data:

SEQ ID NO. 4
subclone 1006, a 500 kb Hind III fragment
1006.seq Length: 400 nt
1 AAATAATCTA AAAATTGGTA GTNCTCCTTC AGATAAAAAT CTTACTTTAA

```
 51  CACCATTCTT TTNAACTNNT TCCGTGTTTC TTTTTCTAAG TCCATCCATA
101  TTTTNAATGA TGTCATCTGC TGTTTTATCT TTTAAATCTA ACACTGAGTG
151  ATAACGGATT TGTAGCACAG GATCAAATCC TTTATGGAAT CCAGTATGTT
201  CAAATCCTAA GTTACTCATT TTATCAAAGA ACCAATCATT ACCAGCATTA
251  CCTGTAATCT CGCCATCATG ATTCAAGTAT TGATATGGTA AATATGGATC
301  GNTATGTAGG TATAGNCAAC GATGTTTTTT AACATATTTT GGATAATTCA
351  TTAAAGNAAA AGTGTACGAG TNCTTGATTT TCATANTCAA TCACTGGACC
```

SEQ ID NO. 5
subclone 1007, a 900 bp Hind III fragment
1007.seq Length: 398 nt

```
  1  TGCGTGAAAT NACTGTATGG CNTGCNATCT GTAAAGGCAC CAAACTCTTT
 51  AGCTGTTAAA TTTGTAAACT TCATTATCAT TACTCCTATT TGTCTCTCGT
101  TAATTAATTT CATTTCCGTA TTTGCAGTTT TCCTATTTCC CCTCTGCAAA
151  TGTCAAAAAT AATAAATCTA ATCTAAATAA GTATACAATA GTTAATGTTA
201  AAACTAAAAC ATAAACGCTT TAATTGCGTA TACTTTTATA GTAATATTTA
251  GATTTTNGAN TACAATTTCA AAAAAAGTAA TATGANCGTT TGGGTTTGCN
301  CATATTACTT TTTTNGAAAT TGTATTCAAT NTTATAATTC ACCGTTTTTC
351  ACTTTTTNCA AACAGTATTC GCCTANTTTT TTTAAATCAA GTAAACTT
```

SEQ ID NO. 6
subclone 1008, a 920 bp EcoR I/ Hind III fragment
1008.seq Length: 410 nt

```
  1  GTAATGACAA ATNTAACTAC AATCGCTTAA AATATTACAA AGACCGTGTG
 51  TNAGTACCTT TAGCGTATAT CAACTTTAAT GAATATATTA AAGAACTAAA
101  CGAAGAGCGT GATATTTTAA ATAAAGATTT AAATAAAGCG TTAAAGGATA
151  TTGAAAAACG TCCTGAAAAT AAAAAAGCAC ATAACAAGCG AGATAACTTA
201  CAACAACAAC TTGATGCAAA TGAGCAAAAG ATTGAAGAAG GTAAACGTCT
251  ACAAGANGAA CATGGTAATG AATTACCTAT CTCTNCTGGT TTCTNCTTTA
301  TCAATCCATT TGANGTTGTT TATTATGCTG GTGGTACATC AAATGCATTC
351  CGTCATTTTN CCGGAAGTTA TGCAGTGCAA TGGGAAATGA TTAATTATGC
401  ATTAAATCAT
```

A partial restriction map of clone pMP33 appears in Fig. 23, with open boxes to represent the percentage of the clone for which DNA sequence has been obtained in one pass.

Analysis of these data reveals identity (> 90%, including sequence ambiguities in first-pass sequence) at both the nucleotide and (predicted) amino acid-level to the *femA* gene of *S. aureus* (Genbank ID M23918; published in Berger-Baechi,B. *et al.*, *Mol. Gen. Genet.* 219 (1989) 263-

269). The nucleotide sequence identities to the Genbank entry indicate that complementing clone pMP33 contains the complete ORF encoding the FemA protein along with the necessary upstream elements for its expression in *S. aureus*. The figure demonstrates the relative positions of the subclones along with the location of the ORF encoding the FemA protein.

<u>Mutant NT64/Clone pMP98: an example of complementing ORFs without direct literature precedent, but identifiable by similarity to genes from other bacteria</u>

The ORF(s) complementing the heat-sensitive phenotype of *S. aureus* mutant NT64 described here were identified by sequencing a subclone of pMP98, an *E. coli/S. aureus* shuttle vector containing a 2.9 kb insert of parental (i.e. wild-type) genomic DNA. The subclone, pMP1038, was generated by EcoRI and HindIII digestion of pMP98 and ligation into pGEM3Zf(+), a commercially available vector (Promega, Inc.) suitable for use in automated fluorescent sequencing applications. Using fluorescently-labelled dye primers (T7 and SP6; ABI, Inc.), a total of 914 bp of sequence from the two edges of the subclone was generated.

SEQ ID NO. 106
subclone 1038, a 2800 bp genomic fragment
1038.sp6 Length: 417 nt

```
  1  GTGATGGATT AAGTCCTAAA TTTNNATTCG CTTTCTTGTC TTTTTAATCT
 51  TTTTCAGACA TTTTATCGAT TTCACGTTTT GTATACTTAG GATTTAAATA
101  GGCATTAATT GTTTTCTTGT CCAAAAATTG ACCATCTTGA TACAAATATT
151  TATCTGTTGG AAATACTTCT TTACTTAAGT NCAATAAACC ATCTTCAAAG
```

201 TCGCCGCCAT TATAACTATT TGCCATGTTA TCTTGTAAAA GTCCTCTTGC
251 CTGGNTTTCT TTAAATGGTA ACAATGTACG NTAGTTATCA CCTTGTACAT
301 TTTTATCCGT TGCAATTTCT TNTACTTGAT TTGAACTATT GTTATGTTTT
351 NAATTATCTT TTCCCAGCCT GGGTCATCCT TATGGTTANC ACAAGCAGCG
401 AGTATAAAGG TAGCTGT

SEQ ID NO. 107
1038.t7 Length: 497 nt
1 TAATGTAGCA ATTACAAGGC CTGAAGAGGT GTTATATATC ACTCATGCGA
51 CATCAAGAAT GTNATTTGGN CGCCCTCAGT CAAATATGCC ATCCAGNTTT
101 TNAAAGGAAA TTCCAGAATC ACTATTAGAA AATCATTCAA GTGGCAAACG
151 ACAAACGGTA CAACCTNNGG CAAAACCTTT TNCTAAACGC GGNTTTTGTC
201 AACGGNCAAC GTCAACGGNN AANCAAGTAT TNTNATCTGN TTGGAATNTT
251 GGTGGCAANG TGGTGCNTAA NGNCNCCGGG GGGAGGCATT GTNNGTAATT
301 TTAACGNGGA NAATGGCTCN NTCGGNCTNG GTNTTATNTT TTATTCACAC
351 AGGGNCGCGN CANGTTTTTT TTGTNGGATT TTTTTCCCCC NTTTTTNAAA
401 AGGNGGGGTN TTNNGGGTGG CTGNTTTANT NGTCTCNGNG TGGNCGTGNN
451 TCATTNNTTT TTTTNTTNNA TCCAAGCCTT NTATGACTTT NNTTGGG

Similarity searches at the nucleotide and (putative) amino acid level reveal sequence identity from the left-most (T7) edge of the clone to the Genbank entry for *pcrA*, a putative helicase from *S. aureus* (Genbank ID M63176; published in Iordanescu,S.M. and Bargonetti,J. *J. Bacteriol.* 171 (1989) 4501-4503) . The sequence identity reveals that the pMP98 clone contains a C-terminal portion of the ORF encoding *pcrA*, but that this ORF is unlikely to be responsible for complementation of the NT64 mutant. The Genbank entry extends 410 bp beyond the 3' end of the *pcrA* gene, and does not predict any further ORFs. Similarity searches with data obtained from the right-most (SP6) edge reveal no significant similarities, indicating that the complementing ORF in pMP98 is likely to be unpublished for *S. aureus*. A partial restriction map of clone pMP98 appears in Fig. 20 (there are no apparent restriction sites for BamH I, EcoR I, or Hind III); the

relative position and orientation of the identified (partial) ORF corresponding to the PcrA protein is indicated by an arrow:

From the preliminary sequence data, the following PCR primers were designed:

pMP98(+): 5' - CTG AAG AGG TGT TAT ATA TCA C - 3'
pMP98(-): 5' - GTG ATG GAT TAA GTC CTA AAT T - 3'

These primers were used to amplify the 2.9 kb genomic DNA fragment in one round of PCR amplification directly from *S. aureus* genomic DNA (parental strain 8325-4). Similar strategies using PCR primers designed from partial sequences can be used for amplifying the genomic sequence (or a cloned genomic sequence) corresponding to the additional complementing clones described below. Additional primers based upon the obtained sequence were designed to generate further DNA sequence data by primer-walking , using the dye terminator strategy (PRISM DyeDeoxy Terminator Kit; ABI, Inc.).

pMP98.b(+): 5' - CTC AGT CAA ATA TGC CAT CCA G - 3'
pMP98.b(-): 5' - CTT TAA ATG GTA ACA ATG TAC G - 3'

The following sequence data were obtained, as depicted in the partial restriction map in Fig. 41:

clone pMP98
SEQ ID NO. 36

pMP98 Length: 2934 nt

1 CATGAAATGC AAGAAGAACG TCGTATTTGT TATGTAGCAA TTACAAGGGC

51	TGAAGAGGTG	TTATATATCA	CTCATGCGAC	ATCAAGAATG	TTATTTGGTC
101	GCCCTCAGTC	AAATATGCCA	TCCAGATTTT	TAAAGGAAAT	TCCAGAATCA
151	CTATTAGAAA	ATCATTCAAG	TGGCAAACGA	CAAACGATAC	AACCTAAGGC
201	AAAACCTTTT	GCTAAACGCG	GATTTAGTCA	ACGAACAACG	TCAACGAAAA
251	AACAAGTATT	GTCATCTGAT	TGGAATGTAG	GTGACAAAGT	GATGCATAAA
301	GCCTGGGGAG	AAGGCATGGT	GAGTAATGTA	AACGAGAAAA	ATGGCTCAAT
351	CGAACTAGAT	ATTATCTTTA	AATCACAAGG	GCCAAAACGT	TTGTTAGCGC
401	AATTTGCACC	AATTGAAAAA	AAGGAGGATT	AAGGGATGGC	TGATTTATCG
451	TCTCGTGTGA	ACGRDTTACA	TGATTTATTA	AATCAATACA	GTTATGAATA
501	CTATGTAGAG	GATAATCCAT	CTGTACCAGA	TAGTGAATAT	GACAAATTAC
551	TTCATGAACT	GATTAAAATA	GAAGAGGAGC	ATCCTGAGTA	TAAGACTGTA
601	GATTCTCCAA	CAGTTAGAGT	TGGCGGTGAA	GCCCAAGCCT	CTTTCAATAA
651	AGTCAACCAT	GACACGCCAA	TGTTAAGTTT	AGGGAATGCA	TTTAATGAGG
701	ATGATTTGAG	AAAATTCGAC	CAACGCATAC	GTGAACAAAT	TGGCAACGTT
751	GAATATATGT	GCGAATTAAA	AATTGATGGC	TTAGCAGTAT	CATTGAAATA
801	TGTTGATGGA	TACTTCGTTC	AAGGTTTAAC	ACGTGGTGAT	GGAACAACAG
851	GTTGAAGATA	TTACCGRAAA	TTTAAAAACA	ATTCATGCGA	TACCTTTGAA
901	AATGAAAGAA	CCATTAAATG	TAGAAKTYCG	TGGTGAAGCA	TATATGCCGA
951	GACGTTCATT	TTTACGATTA	AATGAAGAAA	AAGAAAAAAA	TGATGAGCAG
1001	TTATTTGCAA	ATCCAAGAAA	CGCTGCTGCG	GGATCATTAA	GACAGTTAGA
1051	TTCTAAATTA	ACGGCAAAAC	GAAAGCTAAG	CGTATTTATA	TATAGTGTCA
1101	ATGATTTCAC	TGATTTCAAT	GCGCGTTCGC	AAAGTGAAGC	ATTAGATGAG
1151	TTAGATAAAT	TAGGTTTTAC	AACGAATAAA	AATAGAGCGC	GTGTAAATAA
1201	TATCGATGGT	GTTTTAGAGT	ATATTGAAAA	ATGGACAAGC	CAAAGAAGAG
1251	TTCATTACCT	TATGATATTG	ATGGGATTGT	TATTAAGGTT	AATGATTTAG
1301	ATCAACAGGA	TGAGATGGGA	TTCACACAAA	AATCTCCTAG	ATGGGCCATT
1351	GCTTATAAAT	TTCCAGCTGA	GGAAGTAGTA	ACTAAATTAT	TAGATATTGA
1401	ATTAAGTATT	GGACGAACAG	GTGTAGTCAC	ACCTACTGCT	ATTTTAGAAC
1451	CAGTAAAAGT	AGCTGGTACA	ACTGTATCAA	GAGCATCTTT	GCACAATGAG
1501	GATTTAATTC	ATGACAGAGA	TATTCGAATT	GGTGATAGTG	TTGTAGTGAA
1551	AAAAGCAGGT	GACATCATAC	CTGAAGTTGT	ACGTAGTATT	CCAGAACGTA
1601	GACCTGAGGA	TGCTGTCACA	TATCATATGC	CAACCCATTG	TCCAAGTTGT
1651	GGACATGAAT	TAGTACGTAT	TGAAGGCGAA	GTTAGCACTT	CGTTGCATTA
1701	ATCCAAAATG	CCAAGCACAA	CTTGTTGAAG	GATTGATTCA	CTTTGTATCA
1751	AGACAAGCCA	TGAATATTGA	TGGTTTAGGC	ACTAAAATTA	TTCAACAGCT
1801	TTATCAAAGC	GAATTAATTA	AAGATGTTGC	TGATATTTTC	TATTTAACAG
1851	AAGAAGATTT	ATTACCTTTA	GACAGAATGG	GGCAGAAAAA	AGTTGATAAT
1901	TTATTAGCTG	CCATTCAACA	AGCTAAGGAC	AACTCTTTAG	AAAATTTATT
1951	ATTTGGTCTA	GGTATTAGGC	ATTTAGGTGT	TAAAGCGAGC	CAAGTGTKAG
2001	CAGAAAAATA	TGAAACGATA	GATCGATTAC	TAACGGTAAC	TGAAGCGGAA
2051	TTAGTAGAAT	TCATGATATA	GGTGATAAAG	TAGCGCAATC	TGTAGTTACT
2101	TATTTAGCAA	ATGAAGATAT	TCGTGCTTTA	ATTCCATAGG	ATTAAAAGAT
2151	AAACATGTTA	ATATGATTTA	TGAAGGTATC	CAAAACATCA	GATATTGAAG
2201	GACATCCTGA	ATTTAGTGGT	AAAACGATAG	TACTGACTGG	TAAGCTACAT
2251	CCAAATGACA	CGCAATGAAG	CATCTAAATG	GCTTGCATCA	CCAAGGTGCT
2301	AAAGTTACAA	GTAGCGTTAC	TAAAAATACA	GATGTCGTTA	TTGCTGGTGA
2351	AGATGCAGGT	TCAAAATTAA	CAAAAGCACA	AAGTTTAGGT	ATTGAAATTT
2401	GGACAGAGCA	ACAATTTGTA	GATAAGCAAA	ATGAATTAAA	TAGTTAGAGG
2451	GGTATGTCGA	TGAAGCGTAC	ATTAGTATTA	TTGATTACAG	CTATCTTTAT
2501	ACTCGCTGCT	TGTGGTAACC	ATAAGGATGA	CCAGGCTGGA	AAAGATAATC
2551	AAAAACATAA	CAATAGTTCA	AATCAAGTAA	AAGAAATTGC	AACGGATAAA

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2601 AATGTACAAG GTGATAACTA TCGTACATTG TTACCATTTA AAGAAAGCCA
2651 GGCAAGAGGA CTTTTACAAG ATAACATGGC AAATAGTTAT AATGGCGGCG
2701 ACTTTGAAGA TGGTTTATTG AACTTAAGTA AAGAAGTATT TCCAACAGAT
2751 AAATATTTGT ATCAAGATGG TCAATTTTTG GACAAGAAAA CAATTAATGC
2801 CTATTTAAAT CCTAAGTATA CAAAACGTGA AATCGATAAA ATGTCTGAAA
2851 AAGATAAAAA AGACAAGAAA GCGAATGAAA ATTTAGGACT TAATCCATCA
2901 CACGAAGGTG AAACAGATCG ACCTGCAGKC ATGC
```

From this data, a new ORF in the pMP98 clone was identified as having significant similarity to *lig*, the gene encoding DNA ligase from *E. coli*: (Genbank ID M30255; published in Ishino,Y., *et al.*, *Mol. Gen. Genet.* 204 (1986), 1-7). The revised clone map of pMP98, including the predicted size and orientation corresponding to the putative DNA ligase ORF, is shown in Fig. 41:

The DNA ligase protein from *E. coli* is composed of 671 amino acids; a polypeptide translated from *S. aureus* DNA sequence acquired above matches the C-terminal 82 amino acids of the *E. coli* DNA ligase with a 52% sequence identity and a 67% sequence similarity; this level of similarity is considered significant when comparing proteins from Gram-negative and Gram-positive bacteria. Since the predicted coding region of the *S. aureus* gene for DNA ligase is small enough to be contained within clone pMP98 and the gene for DNA ligase is known to be essential to survival for many bacterial species, NT64 is concluded to contain a *ts* mutation in the gene for DNA ligase.

<u>Mutant NT42/Clone pMP76: an example of complementing ORFs with unknown function</u>

The ORF(s) complementing the temperature-sensitive phenotype of *S. aureus* mutant NT42 described here was identified by sequencing subclones of pMP0076, an *E. coli/S. aureus* shuttle vector containing a 2.5 kb insert of parental (i.e. wild-type) genomic DNA. The subclones, pMP1026 (1.1 kb) and pMP1027 (1.3 kb), were generated by EcoRI and BamHI digestion of the parent clone and ligation into pGEM3Zf(+), a commercially available vector (Promega, Inc.) suitable for double-stranded DNA sequencing applications.

PCR-based methods (PRISM Dye Primer DNA Sequencing Kit; ABI, Inc.) were employed to generate DNA sequence data from the SP6 and T7 promoters of both of the subclones. Primer walking strategies were used to complete the sequence contig. Electrophoresis and detection of fluorescently-labelled DNA sequence ladder on an ABI 373A automated DNA sequencer (ABI, Inc.) yielded the following sequence data:

clone pMP76
SEQ ID NO. 37

```
pMP76  Length: 2515 nt

   1  CSYCGGWACC CGGGGATCCT CTAGAGTCGA TCGTTCCAGA ACGTATTCGA
  51  ACTTATAATT ATCCACAAAG CCGTGTAACA GACCATCGTA TAGGTCTAAC
 101  GCTTCAAAAA TTAGGGCAAA TTATGGAAGG CCATTTAGAA GAAATTATAG
 151  ATGCACTGAC TTTATCAGAG CAGACAGATA AATTGAAAGA ACTTAATAAT
 201  GGTGAATTAT AAAGAAAAGT TAGATGAAGC AATTCATTTA ACACAACAAA
 251  AAGGGTTTGA ACAAACACGA GCTGAATGGT TAATGTTAGA TGTATTTCAA
 301  TGGACGCGTA CGGACTTTGT AGTCCACATG CATGATGATA TGCCGAAAGC
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351  GATGATTATG AAGTTCGACT TAGCATTACA ACGTATGTTA TTAGGGAGAG
401  CCTATACAGT ATATAGTTGG CTTTGCCTCA TTTTATGGTA GAACGTTTGA
451  TGTAAACTCA AATTGTTTGA TACCAAGACC TGAAACTGAA GAAGTAATGT
501  TGCATTTCTT ACAACAGTTA GAAGATGATG CAACAATCGT AGATATCGGA
551  ACGGGTAGTG GTGTACTTGC AATTACTTTG AAATGTTGAA AAGCCGGATT
601  TAAATGTTAT TGCTACTGAT ATTTCACTTG AAGCAATGAA TATGGCTCCG
651  TAATAATGCT GAGAAGCATC AATCACAAAT ACAATTTTTA ACAGGGGATG
701  CATTAAAGCC CTTAATTAAT GAAGGTATCA AKTTGAACGG CTTTGATATC
751  TAATCCMCCA TATATAGATG AAAAAGATAT GGTTACGATG TCTCCMACGG
801  TTACGARATT CGAACCACAT CAGGCATTGT TTGCAGATAA CCATGGATAT
851  GCTATTTATG AATCAATCAT GGAAGATTTA CCTCACGTTA TGGAAAAAGG
901  CAGCCCAGTT GTTTTTGAAA TTGGTTACAA TCAAGGTGAG GCACTTAAAT
951  CAATAATTTT AAATAAATTT CCTGACAAAA AAATCGACAT TATTAAAGAT
1001 ATAAATGGCC ACGATCGAAT CGTCTCATTT AAATGGTAAT TAGAAGTTAT
1051 GCCTTTGCTA TGATTAGTTA AGTGCATAGC TTTTTGCTTT ATATTATGAT
1101 AAATAAGAAA GGCGTGATTA AGTTGGATAC TAAAATTTGG GATGTTAGAG
1151 AATATAATGA AGATTTACAG CAATATCCTA AAATTAATGA AATAAAAGAC
1201 ATTGTTTTAA ACGGTGGTTT AATAGGTTTA CCAACTGAAA CAGTTTATGG
1251 ACTTGCAGCA AATGCGACAG ATGAAGAAGC TGTAGCTAAA ATATATGAAG
1301 CTAAAGGCCG TCCATCTGAC AATCCGCTTA TTGTTCATAT ACACAGTAAA
1351 GGTCAATTAA AAGATTTTAC ATATACTTTG GATCCACGCG TAGAAAAGTT
1401 AATGCAGGCA TTCTGGCCGG GCCCTATTTC GTTTATATTG CCGTTAAAGC
1451 TAGGCTATCT ATGTCGAAAA GTTTCTGGAG GTTTATCATC AGTTGCTGTT
1501 AGAATGCCAA GCCATTCTGT AGGTAGACAA TTATTACAAA TCATAAATGA
1551 ACCTCTAGCT GCTCCAAGTG CTAATTTAAG TGGTAGACCT TCACCAACAA
1601 CTTTCAATCA TGTATATCAA GATTTGAATG GCCGTATCGA TGGTATTGTT
1651 CAAGCTGAAC AAAGTGAAGA AGGATTAGAA AGTACGGTTT TAGATTGCAC
1701 ATCTTTTCCT TATAAAATTG CAAGACCTGG TTCTATAACA GCAGCAATGA
1751 TTACAGAAAT AMTTCCGAAT AGTATCGCCC ATGCTGATTA TAATGATACT
1801 GAACAGCCAA TTGCACCAGG TATGAAGTAT AAGCATTACT CAACCCAATA
1851 CACCACTTAC AATTATTACA GATATTGAGA GCAAAATTGG AAATGACGGT
1901 AAAGATTRKW MTTCTATAGC TTTTATTGTG CCGAGTAATA AGGTGGCGTT
1951 TATACCAAGT GARSCGCAAT TCATTCAATT ATGTCAGGAT GMCAATGATG
2001 TTAAACAAGC AAGTCATAAT CTTTATGATG TGTTACATTC ACTTGATGAA
2051 AATGAAAATA TTTCAGCGGC GTATATATAC GGCTTTGAGC TGAATGATAA
2101 TACAGAAGCA ATTATGAATC GCATGTTAAA AGCTGCAGGT AATCACATTA
2151 TTAAAGGATG TGAACTATGA AGATTTTATT CGTTTGTACA GGTAACACAT
2201 GTCGTAGCCC ATTAGCGGGA AGTATTGCAA AAGAGGTTAT GCCAAATCAT
2251 CAATTTGAAT CAAGAGGTAT ATTCGCTGTG AACAATCAAG GTGTTTCGAA
2301 TTATGTTGAA GACTTAGTTG AAGAACATCA TTTAGCTGAA ACGACCTTAT
2351 CGCAACAATT TACTGAAGCA GATTTGAAAG CAGATATTAT TTTGACGATG
2401 TCGTATTCGC ACAAAGAATT AATAGAGGCA CACTTTGGTT TGCAAAATCA
2451 TGTTTTCACA TTGCATGAAT ATGTAAAAGA AGCAGGAGAA GTTATAGATC
2501 GACCTGCAGG CATGC
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Analysis of the DNA sequence data at the nucleotide level reveals no significant similarity to data in the current release of the Genbank or EMBL databases.

Analysis of the predicted ORFs contained within clone pMP76 reveals a high degree of similarity to two open reading frames identified in *B. subtilis*; "*ipc29D*" and "*ipc31D*" (EMBL entry Z38002). A partial restriction map of pMP76 is depicted in Fig. 42, along with an open box to indicate the percentage of the clone for which DNA sequence has been obtained. The relative orientation and predicted size of the "ipc29D" ORF is indicated by an arrow:

These two ORFs identified from the EMBL entry Z38002 were predicted from genomic sequence data and are denoted as "putative"; no characterization of expression or function of the predicted gene products has been reported in the literature. A similarity has been noted between the predicted Ipc31D-like polypeptide and the SUA5 gene product from yeast (*S. cerevisiae*), but functional characterization still remains to be performed. Hence, the ORFs contained within clone pMP76 represent putative polypeptides of uncertain function, but are known to be responsible for restoring a wild-type phenotype to NT42.

In addition to the illustrative sequences described above, the following sequences of clones complementing heat sensitive mutants of *S. aureus* similarly provide essential genes.

Mutant: NT3
Phenotype: temperature sensitivity
Sequence map: Mutant NT3 is complemented by plasmid pMP27, which contains a 3.9 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in

Fig. 21; open boxes along part of the length of the clone indicate the portions of the clone for which DNA sequence has been obtained (this contig is currently being completed). Database searches at both the nucleic acid and protein levels reveal strong similarity at both the peptide and nucleic acid level to the C-terminal fragment of the SecA protein from *S. carnosus* (EMBL Accession No. X79725) and from *B. subtilis*(Genbank Accession No. D10279). Since the complete SecA ORF is not contained within clone pMP27, SecA is unlikely to be the protein responsible for restoring mutant NT3 to a wild-type phenotype. Further strong peptide-level similarities exist between the DNA sequence of a Taq I subclone of pMP27 and the *prfB* gene, encoding Peptide Release Factor II, of *B. subtilis* (Genbank D10279; published in Pel et al., 1992, *Nucl. Acids Res.* 20:4423-4428). Cross complementation analysis (data not shown) suggests that a mutation in the *prfB* gene is most likely to be responsible for conferring a temperature-sensitive phenotype to mutant NT3 (i.e. it is an essential gene).

DNA sequence data: The following DNA sequence data represents the sequences at the left-most and right-most edges of clone pMP27, using standard M13 forward and M13 reverse sequencing primers, and then extending via primer walking strategies. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP27 (forward and reverse contigs)
SEQ ID NO. 1
pMP27.forward Length: 1739 nt

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  1  CTCGCAGCCG NYAKYCGWAA ATGGTCCAAT GTACTCCATC CATCACTGCA
 51  TCAACCTTAC CTGTTTCTTC GTTCGTACGA TGATCTTTCA CCATTGAGTA
101  TGGATGGAAA ACATATGATC TAATTTGGCT TCCCCAGCCG ATTTCTTTTT
151  GTTCGCCACG AATTTCAGCC ATTTCACGTG CCTGCTCTTC CAATTTTAAT
201  TGATATAATT TAGACTTTAA CATTTTCATA GCTGCTTCAC GGTTTTTAAT
251  TTGAGAACGT TCATTTTGGT TATTAACAAC TATACCTGAG GGGTGGTGGG
301  TAATTCGTAT TGCCGATTCA GTTTTGTTAA TATGCTGACC ACCTGCACCA
351  GAAGCTCTGA ATGTATCAAC TGTAATATCA TCCGGATTGA TTTCAATCTC
401  TATTTCATCA TTATTAAAAT CTGGAATAAC GTCGCATGAT GCAAATGATG
451  TATGACGACG TCCTGATGAA TCAAATGGAG AAATTCGTAC TAGTCGGTGT
501  ACACCTTTTT CAGCTTTTAA ATAACCATAA GCATTATGCC CTTTGATGAG
551  CAATGTTACA CTTTTAATCC CCGCTTCATC CCCAGGTAGA TAATCAACAG
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 601  TTTCAACTTT AAAGCCTTTC TTCTCAACAA TAACGTTGAT ACATTCTAAA
 651  TAGCATATTA GCCCAATCTT GAGACTCCGT GCCACCTGCA CCAGGATGTA
 701  ACTCTAGAAT TGCGTTATTG GCATCGTGAG GCCCATCTAA TAATAATTGC
 751  AATTCGTATT CATCCACTTT AGCCTTAAAA TTAATGACCT CTTGCTCTAA
 801  GTCTTCTTTC ATTTCCTTCA TCAAATTCTT CTTGTAATAA ATCCCAAGTA
 851  GCATCCATGT CATCTACTTC TGCTTGTAGT GTTTTATAAC CATTAACTAT
 901  TGCTTTTAAC GCATTATTTT TATCTATAAT ATCTTGCGCT TTCGTTTGGT
 951  TATCCCAAAA ATTAGGTTCT GCCATCATTT CTTCATATTC TTGAATATTA
1001  GTTTCTTTGT TCTCTAAGTC AAAGAGACCC CCTAATTTGT GTTAAATCTT
1051  GATTATACTT ATCTATATTT CGTTTGATTT CTGATAATTC CATAGCATTC
1101  GCTCCTATTT ATATTTCAAT TCAAGTCATT GATTTGCATC TTTTATAATG
1151  CTAAATTTTA ACATAATTTT GTTAAATAAC AATGTTAAGA AATATAAGCA
1201  CACTGACAAT TAGTTTATGC ATTTATTGTT TAAAAAWGCA GTACATTTAT
1251  GCATCGACAT ATGCCTAAAC CGATTTTTTA AAACTAAGTA CATAACAACG
1301  TTTAACAACT TCTTCACATT TTTTAAAGTA TTTAACGCTT GTAAAATAAA
1351  AAGACTCCTC CCATAACACA AACTATAGGT GTTTAATTGG AAGGAGTTAT
1401  TTTATATCAT TTATTTTCCA TGGCAATTTT TGAATTTTTT ACCACTACCA
1451  CATGGACAAT CATCGTTACG ACCAACTTGA TCGCCTTTAA CGATTGGTTT
1501  CGGTTTCACT TTTTCTTTAC CATCTTCAGC TGAAACGTGC TTCGCTTCAC
1551  CAAACTCTGT TGTTTTTTCA CGTTCAATAT TATCTTCAAC TTGTACTACA
1601  GATTTTAAAA TGAATTTACA AGTATCTTCT TCAATATTTT GCATCATGAT
1651  ATCAAATAAT TCATGACCTT CATTTGATA GTCACGTAAT GGATTTTGTT
1701  GTGCATAAGA ACGTAAGTGA ATACCTTGAC GTAATTGAT
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pMP27.reverse Length: 2368 nt

SEQ ID NO. 2

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   1  CTGCAGGTCG ATCTGCATCT TGATGTTTAT GAAATTCGAG TTGATCTAGT
  51  AATTAAATAA CCAGCTAATA ATGACACTAC ATCAGKAAGA ATAATCCACT
 101  CGTTATGGAA ATACTCTTTA TAGATTGAGG CACCAATTAA AATTAATGTC
 151  AGAATAGTAC CGACCCATTT ACTTCTTGTT ATTACACTAA ATAATACTAC
 201  CAAGACACAT GGAAAGAATG CTGCGCTAAA ATACCATATC ATTCATTTTC
 251  CTCTTTTCTT TTATTTAAAA TGTTCATGGT TGTTTCTCTT AATTCTGTTC
 301  TAGGTATAAA GTTTTCAGTC AACATTTCTG GAATGATATT ATTAATAAAA
 351  TCTTGTACAG ATGCTAAATG GTCAAATTGA ATAATTGTTT CTAGACTCAT
 401  TTCATAAATT TCGAAAAATA ATTCTTCGGG ATTACGKTTT TGTATTTCTC
 451  CAAATGTTTC ATAAAGCAAA TCAATTTTAT CAGCAACTGA AAGTATTTGG
 501  CCTTCTAATG AATCATCTTT ACCTTCTTGC AGTCGTTGCT TATAAACATC
 551  TCTATATTGT AATGGAATTT CTTCTTCAAT AAAGGTCTCT ACCATTTCTT
 601  CTTCAACTTG CGAAAATAAT TTTTTTAATT CACTACTCGC ATATTTAACA
 651  GGTGTTTTTA TATCACCAGT AAACACTTCG GSGAAATCAT GATTTAATGC
 701  TTTTTCATAT AAGCTTTTCC AATTAAYCTT TCTCCATGAT ATTCTTCAAC
 751  TGTTGCTAGA TATTGTGCAA TTTTAGTTAC TTTAAAGGAG TGTGCTGCAA
 801  CATTGTGTTC AAAATATTTA AATTTTCCAG GTAATCTTAT AAGTCTTTCC
 851  ATATCTGATA ATCTTTTAAA ATATTGATGT ACACCCATTT CAATTACCTC
 901  CTCCATTAAT TAATCATAAA TTATACTTTC TTTTTACATA TCAATCAATT
 951  AAATATCATT TAAATATCTT CTTTATATAA CTCTGATTAA ATGATACCAA
1001  AAAATCCTCT CAACCTGTTA CTTAAACAGG CTAAGAGGGT AGTCTTGTCT
1051  TGATATATTA CTTAGTGGAT GTAATTATAT TTTCCTGGAT TTAAAATTGT
1101  TCTTGAAGAT TTAACATTAA ATCCAGCATA GTTCATTTTC AGAAACAGTA
1151  ATTGTTCCMT TTAGGGTTTA CAGATTCAAC AACACCAACA TGTCCATATG
1201  GACCAGCAGC TGTTTGGAAA ATAGCGCCAA CTTCTGGKGT TTTATCTACT
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1251  TTTAAATCCT GCAACTTTTG CTGCGTAATT CCAGTTATTT GCATTGCCCC
1301  ATAAACTTCC TATACTTCTA CCTAATTGTG CACGACGATC GAAAGCATAA
1351  TATGTGCAGT TTCCATAAGC ATATAAGTTT CCTCTGTTAG CAACTGATTT
1401  ATTGTAGTTA TGTGCAACAG GTACAGTTGG TACTGATTTT TGTACTTGAG
1451  CAGGTTTGTA TGCTACATTA ACTGTCTTAG TTACTGCTTG CTTAGGTGCT
1501  TGCTTAACTA CTACTTTTTT AGATGCTTGT TGTACAGGTT GTTTTACTAC
1551  CTTTTTAGCT TGGCTTGCTT TTCTTACTGG TGATTTAACC GCTTTAGTTT
1601  GTTTCACTTT ATTTTGAGGC ACAAGTGAAA TCACGTCACC AGGAAAAATT
1651  AAAGGTGTTA CACCAGGATT GTATTGAATA TAATTGATTC AACGTTAAGT
1701  GATGCTCTTA AAGCAATCTT ATATTAATGA ATCGCCAGCA ACTACTGTWT
1751  AAGTTGTCGG TGATTGCGTT TGTGCTTGAA CATTTGATAC ATAATTATGT
1801  TGAACAGGTG TTTTTACTTG TGTGCCATGT TGTTGTGCAT GTGCKGCATT
1851  ATTTAAAGCK AAAAAAGCTA ACACTGACGA AACCGTCACT GWAAGARART
1901  TTTTCATCTK GCTGTCATTC CTTTGCTGTW AGTATTTTAA GTTATGCAAA
1951  TACTATAGCA CAATACATTT TGTCCAAAAG CTAATTGTTA TAACGANGTA
2001  ATCAAATGGT TAACAANATN AANAGAAGAC AACCGTNTAT CATAGNGGNA
2051  AANGTAGNCA TACCATGNAA TTGAGAACGT TNTCAANAAN TAANTCAATA
2101  CCNTGAAAAT CGCCATAGGN AATATTACNA AATGCACACT GCATATGNTG
2151  NTTTAACAAA CACNACTTTT NANAAATATA NTCTAACTCT ATCTACCGAA
2201  TTGNACTTAA ATATTCATAA ANAAATNATA TTCNAAAATC TAATTTACAA
2251  TTTATTTAGC TACCTTTAAA AAANCNNAAA ACCGACGNCC TTTTAGAGCC
2301  TCGGTTTTTA NATATATNTT AATCGTGCGA CATTGTCTGT TTTNAATNTG
2351  ATTCGACTCT AGNGGATC
```

Mutant: NT5

Phenotype: temperature sensitivity

Sequence map: Mutant NT5 is complemented by plasmid pMP628, which contains a 2.5 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 22. Database searches at both the nucleic acid and protein levels reveal strong similarity between one of the ORFs contained within clone pMP628 and the *zwf* gene from a variety of species, which encodes the Glucose-6-Phosphate Dehydrogenase (G6PD) protein (EC 1.1.1.49). The strongest similarity is demonstrated in the Genbank entry for G6PD (Accession No. M64446; published in Lee, W.T. *et al. J. Biol. Chem.* 266 (1991) 13028-13034.) from *Leuconostoc mesenteriodes*, here abbreviated as "Lme".

DNA sequence data: The following DNA sequence data represents the complete first-pass sequence of pMP628; the sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP628

SEQ ID NO. 3

pMP628 Length: 2494 nt

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   1 AATCATTTTA AATGATTGAT CAAGATGGTA TGGCGAAAGA CCAACGTAAT
  51 CACTTAATTC TTGCAAATTG AAAGGCTCTA ATAAACGATC TTCAATATAA
 101 ACAATTGCCT GTTGTATTTG CTTGATAACG TCCAAAACTT TCACTCCAAT
 151 TAATTCAATC ATTTATTTTT ATTCTACATT ATTTCTATAA ATTATACACC
 201 CATTTGTTCA ATGATTATTA AAATAGTTTT GGGCATTGTA AAATATAATT
 251 TCATAATATA GTCTAGAAAA AAAGCGAATG ATAGAACAAT TGATTTACTT
 301 GATTCGTAAT CAATCCTTGT CATTCGCTCA TTTATTTTTG TTTAACATGT
 351 GCGTTTTAAT TCAATTATTG AATATCGTCC CACCAATGGT TACCATCACG
 401 AGCAAGTAGT AAATCACTTT CTAATGGACC ATTAGTACCT GATTCATAGT
 451 TAGGGAATTC TGGATCAACC ATATTCCATT CATCTTGGAA TTGCATCAAC
 501 AAATTTCCAT GTTGATTTTA ATTCTTCCCA GTGCGTGAAG TTAGTGGCAT
 551 CACCTTTAAG ACAATCAAAT AATAGATTTT CATATGCATC TACAGTATTC
 601 ATTTTATCTT GAGCGCTCAT TGAGTAAGAC AATTGGACAG GTTCTGTTTC
 651 GATACCTTGT GTWTTTTTCT TAGCATTTAR ATGTAAAGAT ACACCTTCAT
 701 TAGGTTGGAT ATTGATTANT AATAGGTTTG AATCTAACAG TTTATCAGTT
 751 TCATAGTATA AGTTCATTGG TACTTCTTTA AATTCAACGA CAACTTGAAT
 801 TGTTTTAGAT TTCATACGTT TACCAGTACG GATATAGAAT GGTACACCAG
 851 CCCATCTAAA GTTATCAATT GTTAATTTAC CTGAAACAAA GGTAGGTGTG
 901 TTAGAGTCAT CTGCAACGCG ATCTTCATCA CGGTATGCTT TAACTTGTTT
 951 ACCATCGATA TAGCCTTCGC CATATTGACC ACGAACAAAG TTCTTTTTAA
1001 CATCTTCAGA TTGGAAATGA CGCAGTGATT TAAGTACTTT TAACTTTCTC
1051 AGCACGGATA TCTTCACTAT TTAAACTAAT AGGTGCTTCC ATAGCTAATA
1101 ATGCAACCAT TTGTAACATG TGGTTTTGCA CCATATCTTT TAGCGCGCCA
1151 CTTGATTCAT AATAACCACC ACGATCTTCA ACACCTAGTA TTTCAGAAGA
1201 TGTAACYYGG ATGTTTGAAA TATATTTGTT ATTCCATAAT GGTTCAAACA
1251 TCGCATTCGC AAAACGTAAT ACCTCGATAT TTTGAACCAT GTCTTTTCCT
1301 AAATAGTGGT CMATACGRTA AATTTCTTCT TCTTTAAATG ATTTACGAAT
1351 TTGATTGTTT AATGCTTCGG CTGATTTTAA ATCACTACCG AATGGTTTTT
1401 CGATAACAAG GCGTTTAAAT CCTTTTGTAT CAGTAAGACC AGAAGATTTT
1451 AGATAATCAG AAATAACGCC AAAGAATTGT GGTGCCATTG CTAAATAGAA
1501 TAGTCGATTA CCTTYTAATT CAAATTGGCT ATCTAATTCA TTACTAAAAT
1551 CTAGTAATTT CTTGATAGCT TTCTTCATTA CTAACATCAT GTCTATGATA
1601 GAAGACATGT TCCATAAACG CGTCAATTTT GTTTGTATCT TTWACGTGCT
1651 TTTGAATTGA TGATTTTAAC TTGATTACGG AAATCATCAT TAGTAATGTC
1701 ACGACGTCCA ATACCGATGA TGGCAATATG TTCATCTAAA TTGTCTTGTT
1751 GGTAGAGATG GAATATTGAT GGAAACAACT TACGATGGCT TAAGTCACCA
1801 GTTGCACCAA AGATTGTGAT TAAACATGGG ATGTGTTTGT TTTTAGTACT
1851 CAAGATTAAA ACCTCAATTC WYMCATTAGA TATATSATTT ATTATKAYMM
1901 GATAATCCAT TTCAGTAGGT CATACMATAT GYTCGACTGT ATGCAGTKTC
1951 TTAAATGAAA TATCGATTCA TGTATCATGT TTAATGTGAT AATTATTAAT
2001 GATAAGTATA ACGTAATTAT CAAAATTTAT ATAGTTATGT CTAACGTTAA
2051 AGTTAGAAAA ATTAACTAGC AAAGACGAAT TTTTAACAGA TTTTGATTCA
2101 AGTATAAATT AAAACTAAAT TGATACAAAT TTTATGATAA AATGAATTGA
2151 AGAAAAGGAG GGGCATATAT GGAAGTTACA TTTTTTGGAA CGAGTGCAGG
2201 TTTGCCTACA AAAGAGAGAA ATACACAAGC AATCGCCTTA AATTTAGAAC
2251 CATATTCCAA TTCCATATGG CTTTTCGACG TTGGTGAAGG TACACAGCAC
```

2301 CAAATTTTAC ATCATGCAAT TAAATTAGGA AAAGTGACAC ATATATTTAT
2351 TACTCATATG CATGGCGATC ATATTTTTGG TTTGCCAGGA TTACTTTCTA
2401 GTCGTTCTTT TCAGGGCGGT GAACAGAAGC CGCTTACATT GGTTGGACCA
2451 AAAGGAATTA AAGCATATGT GGAAATGTCT ATGAATTTAT CAGA

Mutant: NT6

Phenotype: temperature sensitivity

Sequence map: Mutant NT6 is complemented by plasmid pMP33, which contains a 2.3 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 23; open boxes along part of the length of the clone indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and protein levels reveal identity to the *S. aureus* *femA* gene, encoding a protein involved in peptidoglycan crosslinking (Genbank Accession No. M23918; published in Berger-Baechi,B., *et al.*, *Mol. Gen. Genet.* 219, (1989) 263-269). The pMP33 clone contains the complete *femA* ORF (denoted in relative length and direction by an arrow) as well as 5' and 3' flanking DNA sequences, suggesting that it is capable to direct expression of the FemA protein.

DNA sequence data: The following DNA sequence represents sequence data acquired from subclones 1006, 1007 and 1008, using standard sequencing methods and the commercially-available primers T7 and SP6:

subclone 1006, a 500 bp Hind III fragment
SEQ ID NO. 4

1006.sp6 Length: 400 nt
1 AAATAATCTA AAAATTGGTA GTNCTCCTTC AGATAAAAAT CTTACTTTAA
51 CACCATTCTT TTNAACTNNT TCCGTGTTTC TTTTTCTAAG TCCATCCATA
101 TTTTNAATGA TGTCATCTGC TGTTTTATCT TTTAAATCTA ACACTGAGTG
151 ATAACGGATT TGTAGCACAG GATCAAATCC TTTATGGAAT CCAGTATGTT
201 CAAATCCTAA GTTACTCATT TTATCAAAGA ACCAATCATT ACCAGCATTA
251 CCTGTAATCT CGCCATCATG ATTCAAGTAT TGATATGGTA AATATGGATC
301 GNTATGTAGG TATAGNCAAC GATGTTTTTT AACATATTTT GGATAATTCA
351 TTAAAGNAAA AGTGTACGAG TNCTTGATTT TCATANTCAA TCACTGGACC

subclone 1007, a 900 bp Hind III fragment
SEQ ID NO. 5

1007.sp6 Length: 398 nt

```
  1 TGCGTGAAAT NACTGTATGG CNTGCNATCT GTAAAGGCAC CAAACTCTTT
 51 AGCTGTTAAA TTTGTAAACT TCATTATCAT TACTCCTATT TGTCTCTCGT
101 TAATTAATTT CATTTCCGTA TTTGCAGTTT TCCTATTTCC CCTCTGCAAA
151 TGTCAAAAAT AATAAATCTA ATCTAAATAA GTATACAATA GTTAATGTTA
201 AAACTAAAAC ATAAACGCTT TAATTGCGTA TACTTTTATA GTAATATTTA
251 GATTTTNGAN TACAATTTCA AAAAAAGTAA TATGANCGTT TGGGTTTGCN
301 CATATTACTT TTTTNGAAAT TGTATTCAAT NTTATAATTC ACCGTTTTTC
351 ACTTTTTNCA AACAGTATTC GCCTANTTTT TTTAAATCAA GTAAACTT
```

subclone 1008, a 900 bp Hind III fragment
SEQ ID NO. 6

1008.sp6 Length: 410 nt

```
  1 GTAATGACAA ATNTAACTAC AATCGCTTAA AATATTACAA AGACCGTGTG
 51 TNAGTACCTT TAGCGTATAT CAACTTTAAT GAATATATTA AAGAACTAAA
101 CGAAGAGCGT GATATTTTAA ATAAAGATTT AAATAAAGCG TTAAAGGATA
151 TTGAAAAACG TCCTGAAAAT AAAAAAGCAC ATAACAAGCG AGATAACTTA
201 CAACAACAAC TTGATGCAAA TGAGCAAAAG ATTGAAGAAG GTAAACGTCT
251 ACAAGANGAA CATGGTAATG AATTACCTAT CTCTNCTGGT TTCTNCTTTA
301 TCAATCCATT TGANGTTGTT TATTATGCTG GTGGTACATC AAATGCATTC
351 CGTCATTTTN CCGGAAGTTA TGCAGTGCAA TGGGAAATGA TTAATTATGC
401 ATTAAATCAT
```

Mutant: NT8
Phenotype: temperature sensitivity
Sequence map: Mutant NT8 is complemented by plasmid pMP34, which contains a 3.5 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 24. Database searches at both the nucleic acid and protein levels reveal identity to the DNA sequence for the *dfrB* (dihydrofolate reductase [EC 1.5.1.3]; EMBL entry Z16422, published in Dale, G.E. *et al. Antimicrob. Agents Chemother.* 37 (1993) 1400-1405) and *tysY* (thymidylate synthase [EC 2.1.1.45]; EMBL entry X13290, published in Rouch, D.A. *et al. Mol. Microbiol.* 3 (1989) 161-175) genes of *S. aureus*. The relative size and orientations of the genes, along with sequence identities, are depicted as arrows in the restriction map:

DNA sequence data: The following DNA sequence represents data acquired from clone pMP34, starting with M13 forward

and M13 reverse primers and applying primer walking strategies to complete the contig:

clone pMP34
SEQ ID NO. 7

pMP34 Length: 3479 nt

```
   1 AAGCTTCATT AAAAACTTTC TTCAATTTAT CAACATATTC AATGACGTTA
  51 GCATGTGCGA CACCAACGGA YTKSAKKTCA TGATCTCCTA TAAATTCAGC
 101 AATTTCCTTT TTCAAGTATT GGATACTAGA ATTTTGAGTT CTCGCATTGT
 151 GCACAAGCTC TAAGCGACCA TCATCTAGTG TACCAATTGG TTTAATTTTC
 201 ATAAGATTAC CAATCAAACC TTTTGTTTTA CTAATTCTGC CACCTTTAAT
 251 TAATTGATTC AATTGCCCTA TAACTACAAA TAATTTAATG TTTTCTCTTA
 301 AATGATTTAA CTTTTTAACT ATTTCAGAAG TTGAGACACC TTCTTTTACA
 351 AGCTCTACTA GGTGTTGTAT TTGATACCCT AAACCAAAAG AAATAGATTT
 401 TGAATCAATA ACAGTTACAT TAGCATCTAC CATTTGACTT GCTTGGTAAG
 451 CAGTGTTATA TGTACCACTT AATCCTGAAG AAAGATGAAT ACTTATGATT
 501 TCAGAGCCAT CTTTTCCTAG TTCTTCATAA GCAGATATAA ATTCACCTAT
 551 GGCTGGCTGA CTTGTCTTTA CATCTTCATC ATTTTCAATA TGATTAATAA
 601 ATTCTTCTGA TGTAATATCT ACTTGGTCAA CGTATGAAGC TCCTTCAATA
 651 GTTAAACTTA AAGGAATTAC ATGWATGTTG TTTGCTTCTA ARTATTCTTT
 701 AGATAAATCG GATGTTGAGT CTGTTACTAT AATCTGTTTT GTCATGGTCG
 751 TTTTCCCCCT TATTTTTTAC GAATTAAATG TAGAAAGGTA TGTGGAATTG
 801 TATTTTTCTC ATCTAGTTTA CCTTCAACTG AAGAGGCAAC TTCCCAGTCT
 851 TCAAATGTAT AAGGTGGAAA GAACGTATCA CCACGGAATT TACCTTCAAT
 901 AACAGTAATA TACATGTCGT CCACTTTATC AATCATTTCT TCAAATAATG
 951 TTTGCCCTCC AAATATGAAA ACATGGCCCG GTAGTTGGTA AATATCTTCA
1001 ATAGARTGAA TTACATCAAC GCCCTCTACG TTGAAACTTG TATCTGAAGT
1051 AAGTACAACA TTTCGACGAT TCGGTAGTGG TTTACCAATC GATTCAAATG
1101 TCTTACGACC CATTACTAAA GTATGACCTG TTGATAATTT TTTAACATGC
1151 TTCAAATCAT TTGGTAGGTG CCAAGGTAAT TGATTTTCAA AACCAATTAC
1201 TCGTTGCAAG TCATGTGCAA CTAGAATGGA TAAAGTCATA ATTATCCTCC
1251 TTCTTCTATC ATTTCATTTT TTATTACTAA GTTATCTTTA ATTTAACACA
1301 ATTTTTATCA TAAAGTGTGA TAGAAATAAT GATTTTGCAT AATTTATGAA
1351 AACGTTTAAC ACAAAAAAGT ACTTTTTTGC ACTTGAAAAT ACTATGATGT
1401 CATTTKGATG TCTATATGGT TAGCTAAYTA TGCAATGACT ACAMTGCTAT
1451 KGGAGCTTTT ATKGCTGGAT GTGATTCATA GTCAACAATT TCCAMAATCT
1501 TCATAATTTA TGTCGAAAAT AGACTTGTCA CTGTTAATTT TTAATGTTGG
1551 AGGATTGAAG CTTTCACGTG CTAATGGTGT TKCGMATCGC ATCAATATGA
1601 TTTGAATAAA TATGTGCATC TCCAAATGTA TGCACAAATT CACCCACTTC
1651 AAGTCCACAT TTCTTTGGCA ATAAGGTGTG TCAATAAAGC GTAGCYTGCG
1701 ATATTAAATG GCACACCTAA AAAGATATCT GCGCTACGTT GGTATAACTG
1751 GCAACTTAAC TTACCATCTT GGACATAAAA CTGGAACATG GTATGACAAG
1801 GCGGAAGTGC CATTGTATCA ATTTCTGTTG GATTCCATGC AGATACGATG
1851 TGTCGCCTTG AATCTGGATT ATGCTTAATT TGTTCAATTA CTGTTTTAAG
1901 TTGATCAAAA TGATTACCAT CTTTATCAAC CCAATCTCGC CMATTGTTTA
1951 CCATAAACAT TTCCTAAATC CCCGAATTGC TTCGCAAATG TATCATCTTC
2001 AAGAATACGT TGCTTAAATT GTTTCATTTG TTCTTTATAT TGTTCGTTAA
2051 ATTCAGGATC ACTCAATGCA CGATGCCCGA AATCTGTCAT ATCTGGACCT
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2101 TTATACTCGT CTGATTTGAT ATAATTTTCA AAAGCCCATT CGTTCCATAT
2151 ATTATTATTA TATTTTAATA AGTATTGGAT GTTTGTATCT CCTTTAATGA
2201 ACCATAATAA TTCGGTTGCT ACTAATTTAA AAGAAACTTT CTTTGTCGTT
2251 AATAGTGGAA ATCCTTTAGA TAAGTCAAAG CGAAGTTGAT GACCAAATTT
2301 CGAAATCGTA CCTGTATTTG TGCGATCATT TCGTGTATTT CCTATTTCTA
2351 AAACTTCTTC ACAAAGACTG TGATATGCTG CATCAAATGA ATTTCAACAT
2401 ATGCGATAAC ACCTCATTTT CATTATTTAT AGTATGTATA TTTAGTTTGA
2451 TATAACTTAA CTTTATGTAG CATTTTGTTA TCACTCATTT TAGGAATATG
2501 ATATTAATAT CATGAATTCC GTTACTTTAT TTATAAAATG CTGATTAAGT
2551 ACCTACCCCA TCGTAACGTG ATATATGTTT CCAATTGGTA ATTGTTTACC
2601 CAAATCTATA ACTTTAATGC TAAAAAATTT TAAAAAAGAG GTTAACACAT
2651 GATTTGAATA TTATGTTTGA TGTCCTATTA AAACAGTTAA ATTTCTAGAA
2701 AATATAGTTG GTAAAAACGG ACTTTATTTA ACAAATAGAA TACAACTATA
2751 TTCTCTATTT TCAATGACAG ACACCATTTT TAATATTATA AAATGTGTTA
2801 ACCTTTATAT TTATTTATGT GTACTATTTA CAATTTTCGT CAAAGGCATC
2851 CTTTAAGTCC ATTGCAATGT CATTAATATC TCTACCTTCG ATAAATTCTC
2901 TAGGCATAAA ATAAACTAAA TCTTGACCTT TGAATAAAGC ATACGAAGGA
2951 CTAGATGGTG CTTGCTGAAT GAATTCTCGC ATTGTAGCAG TTGCTTCTTT
3001 ATCTTGCCCA GCAAAAACTG TAACTGTATT TGTAGGTCTA TGTTCATTTT
3051 GTGTTGCAAC TGCTACTGCA GCTGGTCTTG CTAATCCAGC TGCACAGCCG
3101 CATGTAGAGT TAATAACTAC AAAAGTAGTG TCATCAGCAT TTACTTGGTT
3151 CATATACTCC GATACTGCTT CGCTCGTTTC TAAACTTGTA AAACCATTTT
3201 GAGTTAATTC GCCACGCATT TGTTGCGCAA TTTCTTTCAT ATAAGCATCA
3251 TAYGCATTCA TATTTAATTC CTCCAATTAA ATTGTTCTGT TTGCCATTTG
3301 TYTCCATACT GAACCAAGYG CTTCAYCTCC GTTTTCAATA TCGAGATATG
3351 GCCATTTCAA TTTGTAATTT AACWTCAAAC GCMTKGTCAK KAATATGGGS
3401 WTTTAGKGCG GGAAGMTGMT YWGCATWACS WTCATSAWAG ATAWACAYAG
3451 CARCAYSCCA CYTWAYGAKT TTMWKTGGA

Mutant: NT12

Phenotype: temperature sensitivity

Sequence map: Mutant NT12 is complemented by pMP37, which contains a 2.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 25. Database searches at both the nucleic acid and peptide levels reveal significant similarities to the protein encoded by the *tagG* gene, an integral membrane protein involved in the assembly of teichoic acid-based structures, from *B. subtilis* (Genbank Accession No. U13832; published in Lazarevic, *et al.*, *Mol. Microbiology*, **16** (1995) 345-355).

DNA sequ nce data: The following DNA sequence data represents the sequence of clone pMP37, using standard M13 forward and M13 reverse sequencing primers and then

completing the sequence contig via primer walking strategies. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP37

SEQ ID NO. 8

```
pMP37  Length: 2875 nt

   1  GTGGTTCCCT GTCATTYTRA TATCCATCAA ACCTTTATTA ATACACGTRG
  51  CTATCGAAGC ATTTTGTAAT TGTATTAATG AAATATGCTT GAGTYCTCTT
 101  TGTAACCGTT CAATCATAGG AATTGTTTGA TCAGTAGAAC CACCATCAAT
 151  ACAAAGGATT CTATAGTGTT CTTTACTCTC AATAGATATT AACAATTGTC
 201  GAATTGTTGC CTCATTATTA CATGTAGGTA TGATTATCGT AAACCTCATT
 251  TTGTCACCAT CTTATCTATA TATTCTGTGA GCTGATGTAA ACTTTTATCA
 301  GTATTATACT TATGCCAATC TTTAAATAAC GGACTTAATA GATGTTCTTT
 351  TTCTTGTATC GTCATTATTA AATCTTCTTC AGTATACACT TTGTAGCTAT
 401  CCGGTATTGC TTTGTAAAAT TGATTCAGGC CTCTCACCTG ATCATATGTT
 451  CCTTCATCAT ACACATAAAA TATAGTTGGA ATATCTAACA AGCTAGCTTC
 501  TATTGGCAGC GAACTATAGT CGCTAATAAT TATATCTGAC ATTAGCATTA
 551  ATGTAGACGT GTCGATTGAA GATACGTCAT CAATGTCTGA ATCTTCAATT
 601  GATGGATGTA ATTTATTAAT CAGTGTATAT CCTGGTAAAC ATTTTTCAAA
 651  ATAAGCTTTA TCAATAGCCC TATTATCTGC TTTATCTTCT CTATATGTTG
 701  GTACATATAA TACCAACTTA TTTGTAATTC CATATTTATC CTTTAACTCT
 751  GCCTTAACCG TTGCTCTATC AGCTGTGTAA TATTTATTAA TTCTCGGAAG
 801  CCCAAAATAC AGCATTTGCT CTTCTGTTGC ACCTAAAGAC TGTTTAAAAC
 851  ATTGTGACAT TTGTTCACAA CCCACTAAGT TAAAAATCCG TCGCTTGATA
 901  AACTTTACGG TACTGCTGAA CCATTGCCTT GTCAGACACA TCGACTTGAT
 951  GATCTGTTAA GCCAAAGTTT TTTAATGCAC CACTTGCATG CCACGTTTGA
1001  ACAATGTGTT TGATTAGAAK TCTTATTATA TCCACCTAGC MATAGGTAAT
1051  AATTATCGAT AATAATCATC TGCGCGCTTT TCAAAGCCTT AATTTGTTTT
1101  ACCAATGTTC GATTAGTCAT TTCTATCACA TCAACATCGT CGCTAAGTTC
1151  AGATAAATAA GGCGCTTGTT TTGGTGTTGT TAAAACAGTT TTCTGATACG
1201  ACGAATTATT TAATGCTTTG ATGATAGGCT TAATATCTTC TGGAAAAGTC
1251  ATCATAAATA CGATATGCGG TTTATCAATC ACTTGAGGSG TAWTCATTTW
1301  AGRAAGTATT CGAACTACCA AATGATAAAA TTTCTTTATT AAAAACGTTC
1351  ATAATAACAC CAACTTAATA TGTTATTTAA CTTAAATTAT AAACAAAAAT
1401  GAACCCCACT TCCATTTATT AATGGTTAGC GGGGTTTCGT CATATAAATA
1451  TATTACAAGA AGTCTGCAAA TTGATCTCTA TATTTCATGT GTWAGTACGC
1501  MCCMATTGCA AAGAAAATGG CAACAATACC GAAATTGTAT AACATTAATT
1551  TCCAATGATC CATGAAATAC CATTCGTGAT ATAAAATTGC TGCACKKTWT
1601  KATTMAKCWR TAMRGTMAAC TRGMTKATAT TTCATCATTK SATGAATTAA
1651  ACCACTGATA CCATGGTTCT TTGGTAGCCA CAAAATTGGT GAAAAGTAAA
1701  ATAATATTCT TAATATTGGC TTGCATTAAC ATTTGTGTAT CTCTAACTAA
1751  CAACACCGAG TGTTGATGTT AATAACGTCA CCGAGGCAGT TAAGAAAAAA
1801  CAAAACGGTA CATATATCAA TAATTGAATG ATATGTATTG ATGGATAAAT
1851  ACCAGTAAAC ATACATGCAA TTATCACAAG TAAAAGTAAG CCTAAATGTC
1901  CATAAAATCT ACTTGTCACA ATATATGTCG GTATTATCGA TAACGGGAAG
1951  TTCATTTTCG ATACTTGATT AAACTTTTGT GTAATTGCTT TAGTACCTTC
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2001  TAAAATACCT TGGTTGATGA AGAACCACAT ACTGATACCA ACCAATAACC
2051  AATAAACAAA AGGTACACCA TGAATTGGTG CATTACTTCT TATTCCTAAT
2101  CCAAAAACCA TCCAGTAAAC CATAATTTGC ATAACAGGGT TAATTAATTC
2151  CCAAGCCACA CCTAAATAGT TACTATGATT GATAATTTTA ACTTGAAACT
2201  GAGCCAGTCT TTGAATTAAA TAAAAGTTCT WTASATGTTC TTTAAAAACT
2251  GTTCCTATTG CTGACATTCC ATTAAACCAC ACTTTCAAAT GTTTAACTAT
2301  TTCTCTAACT TAACTAAATA GTATTATAAT AATTGTTGTA AATACTATCA
2351  CTAWACATGG ATGCTATCAA AATTATTGTC TAGTTCTTTA AAATATTAGT
2401  TTATTACAAA TACATTATAG TATACAATCA TGTAAGTTGA AATAAGTTTA
2451  GTTTTTAAAT ATCATTGTTA TCATTGATGA TTAACATTTT GTGTCAAAAC
2501  ACCCACTCTG ATAATAACAA AATCTTCTAT ACACTTTACA ACAGGTTTTA
2551  AAATTTAACA ACTGTTGAGT AGTATATTAT AATCTAGATA AATGTGAATA
2601  AGGAAGGTCT ACAAATGAAC GTTTCGGTAA ACATTAAAAA TGTAACAAAA
2651  GAATATCGTA TTTATCGTAC AAATAAAGAA CGTATGAAAG ATGCGCTCAT
2701  TCCCAAACAT AAAAACAAAA CATTTTTCGC TTTAGATGAC ATTAGTTTAA
2751  AAGCATATGA AGGTGACGTC ATAGGGCTTG TTGGCATCAA TGGTTCCGGC
2801  AAATCAACGT TGAGCAATAT CATTGGCGGT TCTTTGTCGC CTACTGTTGG
2851  CAAAGTGGAT CGACCTGCAG TCATA
```

Mutant: NT14

Phenotype: temperature sensitivity

Sequence map: Mutant NT14 is complemented by plasmid pMP40, which contains a 2.3 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 26 (no Eco RI, Hind III, Bam HI or Pst I sites are apparent); open boxes along part of the length of the clone indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and protein levels reveal identity to the Staph. aureus femB gene, encoding a protein involved in peptidoglycan crosslinking (Genbank Accession No. M23918; published in Berger-Baechi,B., et al., Mol. Gen. Genet. 219, (1989) 263-269). The pMP40 clone contains the complete FemB ORF (denoted in relative length and direction by an arrow) as well as 5′ and 3′ flanking DNA sequences, suggesting that it is capable to direct expression of the FemB protein; the relation of the femA gene is also depicted to demonstrate the extent of identity between the clone and the Genbank entry.

DNA sequence data: The following DNA sequence data represents the sequences at the left-most and right-most

edges of clone pMP40 obtained with the standard DNA sequencing primers T7 and SP6, and can be used to demonstrate identity to part of the published sequence (Genbank No. M23918):

SEQ ID NO. 9

1015.t7 LENGTH: 453 nt

```
  1  CTTAAAATAT TACAAAGACC GTGTGTNAGT ACCTTNAGCG TATATcAaCT
 51  TTAATGAATA TATTAAAGAA CTAAACGAAG AGCGTGATAT TTTAAATAAA
101  GATTTAAATA AAGCGTTAAA GGATATTGAA AAACGTCCTG AAAATAAAAA
151  AGCACATAAC AAGCGAGATA ACTTACAACA ACAACTTGAT GCAAATgAGC
201  AAAAGATTGA NGACGGTAAA CGTCTACAAG ANGANCATGG TAATGNTTTA
251  CCTATCTCTC CTGGTTTCTC CTTTATCAAT CCNTTTGANG TTGTTTATTA
301  TGCTGGTGGT ACATCAAATG CNTTCCGTCA TTTTNCCGGA NGTTATGCNG
351  TGCAATGGGA AATGNTTAAT TTTGCATTAA ATCATGGCAT TGNCCGTTAT
401  AATTNCTATG GTGTTAGTGG TNAATTTNCA GNAGGTGCTG AAGATGCTGG
451  TGT
```

SEQ ID NO. 10

1015.sp6 LENGTH: 445 nt

```
  1  ATGCTCAGGT CGATCATACA TCTATCATCA TTttAATTTC TAAAATACAA
 51  ACTGAATACT TTCCTAGAaT NTNaNACAGC AATCATTGCT CATGCATTTA
101  ATAAATTaCA ATTAGACAAA TATGACATTT gATATCACAC ACTTGCAAAC
151  ACACACATAT ATAATCAGAC ATAAATTGTT ATGCTAAGGT TTATTCACCA
201  AAANTATAAT ACATATTGGC TTGTTTTGAG TCATATTGNN TGANTTANAA
251  NGTATACTCA ACTCANTCAT TTNCAAATNG GTTGTGCAAT TCNTATTTNT
301  NTTTCTTGCA ATCCCTTGTT AAACTTGTCA TTTNATATAT CATTNTTCGG
351  GGCTTTATTA AAANNCATNT NNNACNGNGC CTATNGNNTC NNTNACTATN
401  NGCCCTAACA TCATTTTCNT CTNTTTCTTA TTTTTTACGG GATTT
```

Mutant: NT15

Phenotype: temperature sensitivity

Sequence map: Mutant NT15 is complemented by plasmid pMP102, which contains a 3.1 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig.27; open boxes along part of the length of the clone indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and protein levels reveal strong identity at both the peptide and nucleic acid level to the SecA protein from *S. carnosus* (Genbank Accession No. X79725; submitted in 1994, unpublished as of 1995); the relative size and location of the *secA* gene predicted from similarity to the *S. carnosus* gene is depicted below by an arrow. The SecA protein is

involved in the protein secretory pathway and serves an essential cellular function.

DNA sequence data:

clone pMP102

SEQ ID NO. 11

pMP102.forward Length: 719 nt

```
  1 GATCRAGGAG ATCAAGAAGT GTTTGTTGCC GAATTACAAG AAATGCAAGA
 51 AACACAAGTT GATAATGACG CTTACGATGA TAACGAGATA GAAATTATTC
101 GTTCAAAAGA ATTCAGCTTA AAACCAATGG ATTCAGAAGA AGCGGTATTA
151 CAAATGAATC TATTAGGTCA TGACTTCTTT GTATTCACAG ACAGAGAAAC
201 TGATGGAACA AGTATCGTTT ACCGCCGTAA AGACGGTAAA TATGGCTTGA
251 TTCAAACTAG TGAACAATAA ATTAAGTTTA AAGCACTTGT GTTTTTGCAC
301 AAGTGCTTTT TTATACTCCA AAAGCAAATT ATGACTATTT CATAGTTCGA
351 TAATGTAATT TGTTGAATGA AACATAGTGA CTATGCTAAT GTTAATGGAT
401 GTATATATTT GAATGTTAAG TTAATAATAG TATGTCAGTC TATTGTATAG
451 TCCGAGTTCG AAAATCGTAA AATATTTATA ATATAATTTA TTAGGAAGTT
501 ATAATTGCGT ATTGAGAATA TATTTATTAG TGATAAACTT GTTTGACACA
551 GAATGTTGAA TGAATTATGT CATAAATATA TTTATATTGA TCTACCAATG
601 AGTAAATAAN TATAATTTCC TAACTATAAA TGATAAGANA TATGTTGTNG
651 GCCCAACAGT TTTTTGCTAA AGGANCGAAC GAATGGGATT TTATCCAAAA
701 TCCTGATGGC ATAATAAGA
```

SEQ ID NO. 12

pMP102.reverse Length: 949 nt

```
  1 CTTTACCATC TTCAGCTGAA ACGTGCTTCG CTTCACCAAA CTCTGTTGTT
 51 TTTTCACGTT CAATATTATC TTCAACTTGT ACTACAGATT TTAAAATGAA
101 TTTACAAGTA TCTTCTTCAA TATTTTGCAT CATGATATCA AATAATTCAT
151 GACCTTCATT TTGATAGTCA CGTAATGGAT TTTGTTGTGC ATAAGAACGT
201 AAGTGAATAC CTTGACGTAA TTGATCCATT GTGTCGATAT GATCAGTCCA
251 ATGGCTATCA ATAGAACGAA GTAAAATCAT ACGCTCAAAC TCATTCATTT
301 GTTCTTCTAA GATATCTTTT TGACTTTGAT ATGCTGCTTC AATCTTAGCC
351 CAAACGACTT CGAAAATATC TTCAGCATCT TTACCTTTGA TATCATCCTC
401 TGTAATGTCA CCTTCTTGTA AGAAGATGTC ATTAATGTAG TCGATGAATG
451 GTTGATATTC AGGCTCGTCA TCTGCTGTAT TAATATAGTA ATTGATACTA
501 CGTTGTAACG TTGAACGTAG CATTGCATCT ACAACTTGAG AGCTGTCTTC
551 TTCATCAATA ATACTATTTC TTTCGTTATA GATAATTTCA CGTTGTTTAC
601 GTAATACTTC ATCGTATTCT AAGATACGTT TACGCGCGTC GAAGTTATTA
651 CCTTCTACAC GTTTTTGTGC TGATTCTACA GCTCTTGATA CCATTTTTGA
701 TTCAATTGGT GTAGAGTCAT CTAAACCTAG TCGGCTCATC ATTTTCTGTA
751 AACGTTCAGA ACCAAAAACGA AATCATTAAT TCATCTTGTA ATGATAAATA
801 GAAGCGACTA TCCCCTTTAT CACCTTGACG TCCAGAACGA CCACGTAACT
851 GGTCATCAAT ACGACGAAGA TTCATGTCGC TCTGTACCTA TTACTGCTAA
901 ACCGCCTAAT TCCTCTACGC CTTCACCTAA TTTGATATCT GTACCACGA
```

SEQ ID NO. 13

pMP102.subclone Length: 594 nt

```
  1 GGGGATCAAT TTANAGGACG TACAATGCCA GGCCGTCGTT NCTCGGAAGG
 51 TTTACACCAA GCTATTGAAG CGAGGAAAGG CGTTCAAATT CAAAATGAAA
101 TCTAAAACTA TGGCGTCTAT TACATTCCAA AACTATTTCA GAATGTACAA
151 TAAACTTGCG GGTATGACAG GTACAGCTAA AACTGAAGAA GAAGAATTTA
201 GAAATATTTA TAACATGACA GTAACTCAAA TTCCGACAAA TAAACCTGTG
251 CAACGTAACG ATAAGTCTGA TTTAATTTAC ATTAGCCAAA AAGGTAAATT
301 TGATGCAGTA GTAGAAGATG TTGTTGAAAA ACACAAGGCA GGGCAACCMG
351 TGCTATTAGG TACTGTTGCA GTTGAGACTT CTGTATATAT TTCAAATTTA
401 CTTAAAAAAC GTGGTATCCG TCATGATGTG TTAAATGCGA RAAATCATGA
451 MCGTGAAGCT GAAATTGTTG CAGGCGCTGG RCAAAAAGGT GCCGTTACTA
501 TTGCCACTAM CATGGCTGGT CGTGGTACAG ATATCAAATT AGGTGAAGGC
551 GTTANAANGA AATTAGGCGG TTTANCCAGT AATANGTTCA GAAG
```

Mutant: NT16

Phenotype: temperature sensitivity

Sequence map: Mutant NT16 is complemented by plasmid pMP44, which contains a 2.2 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 28. Database searches at both the nucleic acid and protein levels reveal significant similarity at the peptide level to an ORF (orf3) of unknown function in the serotype "A" capsulation locus of *H. influenzae* (Genbank Accession No. Z37516); similarity also exists at the protein level to the *tagB* gene of *B. subtilis* (Genbank Accession No. X15200), which is involved in teichoic acid biosynthesis. Based upon the peptide level similarities noted, it is possible that the ORF(s) contained within this clone are involved in some aspect of membrane biogenesis, and should make an excellent screening target for drug development. No significant similarities are observed at the nucleic acid level, strengthening the stance that clone pMP44 represents a novel gene target(s).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP44, starting with standard M13 forward and M13 reverse sequencing primers. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP44

SEQ ID NO. 14

pMP44 Length: 2192 nt

```
   1 GCATGMCTGC AGGTCGATCY SYTGAACAGT CATCAACTAC AACCACTTCA
  51 AATTCAGTTT TCGGAAAATC TTGTTTCGCA AGGCTATTAA GTAATTCTGT
 101 TATATACTTT TCTGAATTGT ATGTTGGAAC TATTACTGAA AATTTCATCA
 151 TTATACCTCT CCCACTTTGA CTACTATATA AACTTAGCTA CCAAATAAAT
 201 TTCTGACTAA ACGCTCACTT GATCGGCCAT CTTGATATTT AAAATGTTTA
 251 TCTAAGAATG GAATGACTTT TTCTCCTTCA TAATCTTCAT TGTCCAAGGC
 301 GTCCATTAAT GCGTCAAATG ATTGCACAAT TTTACCTGGA ACAAATGATT
 351 CATATGGTTC ATAAAAATCA CGCGTCGTAA TATAATCTTC TAAATCAAAT
 401 GCATAGAAAA TCATTGGCTT TTTAAATACT GCATATTCAT ATATTAAAGA
 451 TGAATAGTCA CTAATTAATA AATCTGTTAT GAACAGTATA TCATTAACTT
 501 CTCTAAAGTC AGAAACGTCA ACAAAATATT GTTTATGTTT GTCTGCAATA
 551 TTAAGTCTAT TTTTCACAAA TGGATGCATT TTAAATAATA CAACCGCGTT
 601 ATTTTTTTCG CAATATCTTG CTAAACGTTC AAAATCAATT TTGAAAAATG
 651 GGTAATGTGC TGTACCATGA CCACTACCTC TAAATGTTGG TGCGAAAAGA
 701 ATGACTTTCT TACCTTTAAT AATTGGTAAT TCATCTTCCA TCTCTTGTTT
 751 GATCTGTGTC GCATAAGCTT CATCAAATAG TACATCAGTA CGTTGGGAAC
 801 ACCTGTAGGC ACTACATTTT TCTCTTTAAT ACCAAATGCT TCAGCGTAGA
 851 ATGGAATATC GGTTTCAAGA TGATACATAA GCTTTTGTAT AAGCTACGGA
 901 TGATTTAATG AATCAATAAA TGGTCCACCC TTTTTACCAG TACGACTAAA
 951 GCCAACTGTT TTAAAGGCAC CAACGGCATG CCATACTTGA ATAACTTCTT
1001 GAGAACGTCT AAAACGCACT GTATAAATCA ATGGGTGAAA GTCATCAACA
1051 AAGATGTAGT CTGCCTTCCC AAGTAAATAT GGCAATCTAA ACTTGTCGAT
1101 GATGCCACGT CTATCTGTAA TATTCGCTTT AAAAACAGTG TGAATATCAT
1151 ACTTTTTATC TAAATTTTGA CGTAACATTT CGTTATAGAT GTATTCAAAG
1201 TTTCCAGACA TCGTTGGTCT AGAGTCTGAT GTGAACAACA CCGTATTCCC
1251 TTTTTTCAAG TGGAAAAATT TCGTCGTATT AAATATCGCT TTAAAAATAA
1301 ATTGTCTTGT ATTAAATGAT TGTTTGCGGA AATACTTACG TAATTCTTTA
1351 TATTTACGRA CGATATAAAT ACTTTTAAMT TCCCGGAGTC GTTACAACAA
1401 CATCAAGGAC AAATTCATTA ACATCGCTAG AAATTTCAGG TGTAACAGTA
1451 TAAACCGTTT TCTTTCGAAA TGCCGCCTTT TCTAAATTCT TTTAGGTAAG
1501 TCTGCAATAA GAAATTGATT TTACCATTTT GTGTTTCTAA TTCGYTGTAT
1551 TCTTCTTCTT GTTCTGGCTT TAGATTTTGA TATGCATCAT TAATCAACAT
1601 CTGGGTTTAA CTGTGCAATA TAATCAAGTT CTTGCTCATT CACTAATAAG
1651 TACTTATCTT CAGGTAAGTA ATAACCATTA TCTAAGATAG CTACATTGAA
1701 ACGACAAACG AATTGATTCC CATCTATTTT GACATCATTC GCCTTCATTG
1751 TACGTGTCTC AGTTAAATTT CTTAATACAA AATTACTATC TTCTAAATCT
1801 AGGTTTTCAC TATGTCCTTC AACGAATAAC TGAACACGTT CCCAATAGAT
1851 TTTAYCTATA TATATCTTAC TTTTAACCAA CGTTAATTCA TCCTTTTCTA
1901 TTTACATAAT CCATTTTAAT ACTGTTTTAC CCCAAGATGT AGACAGGTCT
1951 GCTTCAAAAG CTTCTGTAAG ATCATTAATT GTTGCAATTT CAAATTCTTG
2001 ACCTTTTAAA CAACGGCTAA TTTATCTAAC AATATCTGGG TATTGAATGT
2051 ATAAGTCTAA CAACATCTTG GAAATCTTTT GAACCACTTC GACTACTACC
2101 AATCAACGTT AGTCCTTTTT CCAATACTAG AACGTGTATT AACTTCTACT
2151 GGGAACTCAC TTACACCTAA CAGTGCAATG CTTCCTTCTG GT
```

Mutant: NT17

Phenotype: temperature sensitivity

Sequence map: Mutant NT17 is complemented by plasmid pMP45, which contains a 2.4 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 29. Database searches at both the nucleic acid and protein levels reveal a strong similarity to the product of the *apt* gene, encoding adenine phosphoribosyl transferase (EC 2.4.2.7) from *E. coli* (Genbank Accession No. M14040; published in Hershey, H.V. *et al. Gene* **43** (1986) 287-293).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking into clone pMP45, starting with standard M13 forward and M13 reverse sequencing primers. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP45

SEQ ID NO. 15

pMP45 Length: 2431 nt

```
   1  ATGCAGGTCG ATCNCCTNGT TTATTCNGNT TCATCATTTT CCGATAAATA
  51  CTGTAAATAT GNNTAGGTCT ACCATTTATA TCGCCTTCGA TATTCATTCG
 101  GTCCATTTCA GTACGTATTC TATCAATAGC CGTTTCGATA TACGCTTCAC
 151  GTTCACTACG TTTCTTCTTC ATTAAATTGA CTATTCTAAA ATATTGCACA
 201  TTATCAATAT AACGAAGAGC CGKATCTTCT AGTTCCCATT TGATTGTATT
 251  AATACCAAGA CGATGTGCTA ATGGTGCATA AATTTCTAAT GTTTCTCGAG
 301  AAATTCTAAT TTGKTTTTCG CGCGGSATGG STTTCAAGGT ACGCATATTA
 351  TGTAATCTGT CTGCTAATTT CAMCAAAATT ACGCGTACAT CTTTGGCAAT
 401  CGCAATAAAT AACTTGSGAT GATTTTCAGC TTGTTGTTCT TCTTTTGAGC
 451  GGTATTTTAC TTTTTTAAGC TTCGTCACAC CATCAACAAT TCGAGCAACT
 501  TCTTCATTGA ACATTTCTTT TACATCTTCA AATGTATACG GTGTATCTTC
 551  AATTACATCA TGCAAAAAAC CTGCGACAAT CGTCGGTCCG TCTAATCGCA
 601  TTTCTGTTAA AATACCTGCA ACTTGTATAG GATGCATAAT GTATGGTAAT
 651  CCGTTTTTTC GGAACTGACC TTTATGTGCT TCATAAGCAA TATGATAGCT
 701  TTTTAAAACA TACTCATATT CATCTGCTGA CAAATATGAT TTTGCTTTGT
 751  GAAGAACTTC GTCTGCACTA TATGGATATT CGTTGTTCAT TATATGATAC
 801  ACCCCATTCA TATTTATTAC TTCGCCTTTA AACAATGGAT TTAGGTACTC
 851  TTGTTGAATA GTATTTGTCC CACACCAATC ATACGTCCGT CGACGATAAA
 901  TATTTATCCT GTCGTGCATT AATCGTAATA TTAATTTTAC TTGAGCGAGT
 951  TTAATTTGTA TACTATTCCT ACTTTTAAAA CTTTTACAAA AATTCGACCT
1001  AAATCTACTG TTTCATTTTT TAAATATTAG TTCTATGATA CTACAATTTA
1051  TGARATAAAT AAACGAWGTT ATTAAGGTAT AATGCTCMAT CATCTATCAT
```

1101 TTTCAGTAAA TAAAAAATCC AACATCTCAT GTTAAGAAAA CTTAAACAAC
1151 TTTTTTAATT AAATCATTGG TYCTTGWACA TTTGATRGAA GGATTTCATT
1201 TGATAAAATT ATATTATTTA TTATTCGTCG TATGAGATTA AACTMATGGA
1251 CATYGTAATY TTTAAWAKTT TTCMAATACC AWTTAAAWKA TTTCAATTCA
1301 AATTATAAAW GCCAATACCT AAYTACGATA CCCGCCTTAA TTTTTCAACT
1351 AATTKTATKG CTGYTCAATC GTACCACCAG TAGCTAATAA ATCATCTGTA
1401 ATTRRSACAG TTGACCTGGK TTAATTGCAT CTTKGTGCAT TGTYAAAACA
1451 TTTGTACCAT ATTCTAGGTC ATAACTCATA ACGAATGACT TCACGAGGTA
1501 ATTTCCCTTC TTTTCTAACA GGTGCAAAGC CAATCCCCAT KGAATAAGCT
1551 ACAGGACAGC CAATGATAAA GCCAACGSGC TTCAGGTCCW ACAACGATAT
1601 CAAACATCTC TGTCTTTTGC GTATTCWACA ATTTTATCTG TTGCATAGCC
1651 ATATGCTTCA CCATTATCCA TAATTGTAGT AATATCCTTG AAACTAACAC
1701 CTGGTTTCGG CCAATCTTGA ACTTCTGATA CGTATTGCTT TAAATCCATT
1751 AATATTTCCT CCTAAATTGC TCACGACAAT TGTGACTTTA TCCAATTTTT
1801 TATTTCTGAA AAATCTTGAT ATAATAATTG CTTTTCAACA TCCATACGTT
1851 GTTGTCTTAA TTGATATACT TTGCTGGAAT CAATCGATCT TTTATCAGGT
1901 TGTTGATTGA TTCGAATTAA ACCATCTTCT TGTGTTACAA ATTTTAAGTC
1951 TAAGAAAACT TTCAACATGA ATTTAAGTGT ATCTGGTTTC ACACTTAAAT
2001 GTTGACACAA TAACATACCC TCTTTCTGGA TATTTGTTTC TTGTTTAGTT
2051 ATTAATGCTT TATAACACTT TTTAAAAATA TCCATATTAG GTATACCATC
2101 GAAGTAAATC GAATGATTAT GTTGCAAAAC TATAKAAAGW TGAGAAAATT
2151 GCAGTTGTTG CAAGGAATTA GACAAGTCTT CCATTGACGT TGGTAAATCT
2201 CTTAATACTA CTTTATCAGT TTGTTGTTTA ATTTCTTCAC CATAATAATA
2251 TTCATTCGCA TTTACTTTAT CACTTTTAGG ATGAATAAGC ACGACAATAT
2301 TTTCATCATT TTCTGTAAAA GGTAAACTTT TTCGCTTACT TCTATAATCT
2351 AATATTTGCT GTTCATTCAT CGCAATATCT TGAATAATTA TTTGCGGTGA
2401 TTGATTACCA TTCCATTCGT TGATTTGAAC A

Mutant: NT18
Phenotype: temperature sensitivity
Sequence map: Mutant NT18 is complemented by pMP48, which contains a 4.7 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 30, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained; the sequence contig will be completed shortly. Database searches at both the nucleic acid and peptide levels reveal a strong peptide-level similarity to the *ureD* gene product, encoding a putative regulatory protein with strong similarities to the phosphomannomutase and the phosphoglucomutase from *E. coli*. The right-most sequence contig from the diagram below is responsible for complementing mutant NT102, described later; however, the full pMP48 clone described here is required for complementing mutant NT18. Based upon genomic

organization and peptide-level similarities, it is highly likely that mutants NT18 and NT102 represent two different proteins in the same biochemical pathway.

DNA sequence data: The following DNA sequence data represents the sequence obtained from clone pMP48, starting with standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to augment the sequence contigs. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP48

SEQ ID NO. 16

pMP48.forward Length: 2018 nt

1	GCATCAGTTG	GTACTTTAAA	TAAATGTGCA	GTACCAGTCT	TAGCAACATT
51	TACAGTTGCT	AATTCAGTAT	TTTTCTTAGC	ATCTTTAATA	ACTAAATTTG
101	TTGCACCTTG	CTTACTATTC	GTTTGCATAG	TAGTAAAGTT	AATAATTAAT
151	TCTGAATCTG	GTTTTACATT	TACAGTTTTT	GAAATACCGT	TAAAGTTACC
201	ATGATCTGTA	GAATCATTTG	CATTCACACG	ACCTAATGCA	GCCACGTTTC
251	CTTTAGCTTG	ATAGTTTTGA	GGGTTATTCT	TATCAAACAT	ATCGCTTCGT
301	CTTAATTCTG	AGTTAACGAA	ACCAATCTTA	CCGTTGTTAA	TTAATGAATA
351	ACCATTTACT	TTATCTGTAA	CAGTTACAGT	TGGATCCTGT	CTATTCTCAT
401	CTGTTGATAT	GGCAGGATCA	TCAAATGTTA	ATGTCGTATT	AATACTGCCT
451	TCACCAGTAT	TGCTAGCATT	TGGATCTTGA	GTTTGTGCGT	TTGCTGCTAC
501	AGGTGCTGCT	GGTTGCGCTG	CTGCTGGANC	ATTCGCTGGC	TGTGTTTGAT
551	TTGCCGGTGT	TGCATTATTA	TWAGGTGTTG	CTTGGTTATT	TCCTTGACCT
601	GCTTGGTWTG	CCGGTGTTGC	TTGATTTCCA	GGTTGTGCAT	GTGCAACGTT
651	ATTCGGATCA	GCTTGATCAC	CTTGTCCAGC	TGGTTGTGTA	TTTGGTTGTG
701	CTGCTCCTCC	TGCTGGATTA	GCCTGTCCAC	CTTGGTTTGC	TGGTTGTACT
751	GCTGGTTGTC	CTTGGTTGGC	AGGTGCAGCT	GGCTGTGCTG	TAGGATTAGC
801	TTGAGCACCA	GCATTTGCGT	TAGGCTGTGT	ATTGGCATCA	GCTGGTTGTG
851	CTGGTTGATT	TTGTGCAGGC	TGATTTTGCT	CTGCTGCAKA	CGCTGTTGTC
901	GGGTTAGTAG	ATATAAAAGT	AACAGTGGCA	ATTAAAGCTG	AAAAAATACC
951	GACATTAAAT	TTTCTGATAC	TAAATTTTTG	TTGTCTGAAT	AAATTCATTA
1001	AGTCATCCTC	CTGGTTGATT	ATTCTCGCTG	TTAAATGATT	TCACTTAATC
1051	AACTGTTAAG	ATAAGTAGTA	GCATCTGCGT	TAAAAACACA	AAGCAACTCT
1101	ATCTAATTAA	AATTAATTTT	ATCATCATTA	TATATTGAGT	ACCAGTGTAT
1151	TTTATATTAC	ATATTGATTA	CTTTGTTTTT	ATTTTGTTTA	TATCATTTTA
1201	CGTTTGTACT	ATAAATTATT	TCTACAAACA	CAAAAAACCG	ATGCATACGC
1251	ATCGGCTCAT	TTGTAATACA	GTATTTATTT	ATCTAATCCC	ATTTTATCTT
1301	GAACCACATC	AGCTATTTGT	TGTGCAAATC	TTTCAGCATC	TTCATCAGTT
1351	GCTGCTTCAA	CCATGACACG	AACTAATGGT	TCTGTTCCAG	AAGGTCTTAC
1401	TAAAATTCGA	CCTTCTCCAT	TCATTTCTAC	TTCTACTTTA	GTCATAACTT
1451	CTTTAACGTC	AACATTTTCT	TCAACACGAT	ATTTATCTGT	TACGCGTACG
1501	TTAATTAATG	ATTGTGGATA	TTTTTTCATT	TGTCCAGCTA	ATTCACTTAG

1551 TGATTTACCA GTCATTTTTA TTACAGAAGC TAATTGAATA CCAGTTAATA
1601 AACCATCACC AGTTGTATTG TAATCCAYCA TAACGATATG TCCARATKGT
1651 TCTCCACCTA AGTTATAATT ACCGCGAMGC ATTTCTTCTA CTACATATCT
1701 GTCGCCAACT TTAGTTTTAT TAGATTTAAT TCCTTCTTGT TCAAGCGCTT
1751 TGTAAAAACC TAAATTACTC ATAACAGTAG AAAACGAATC ATGTCATTAT
1801 TCAATTCTTG ATTTTATGC ATTTCTTGAC CAATAATAAA CATAATTTGG
1851 TCACCGTCAA CGATTTGACC ATTCTCATCT ACTGCTATGA TTCTGTCTCC
1901 ATCGCCGTCA AATGCTAACC CAAAATCACT TTCAGTTTCA ACTACTTTTT
1951 CAGCTAATTT TCAGGATGTG TAAAGCCACA TTTCTCATTG ATATTATATC
2001 CATCAGGGAC TACATCCA

SEQ ID NO. 17

pMP48.reverse Length: 2573 nt

1 ATTCGAGCTC GGTACCCGKG GATCCTSYAG AGTCGATCCG CTTGAAACGC
51 CAGGCACTGG TACTAGAGTT TTGGGTGGTC TTAGTTATAG AGAAAGCCAT
101 TTTGCATTGG AATTACTGCA TCAATCACAT TTAATTTCCT CAATGGATTT
151 AGTTGAAGTA AATCCATTGA TTGACAGTAA TAATCATACT GCTGAACAAG
201 CGGTTTCATT AGTTGGAACA TTTTTTGGTG AAACTTTATT ATAAATAAAT
251 GATTTGTAGT GTATAAAGTA TATTTTGCTT TTTGCACTAC TTTTTTTAAT
301 TCACTAAAAT GATTAAGAGT AGTTATAATC TTTAAAATAA TTTTTTTCTA
351 TTTAAATATA TGTTCGTATG ACAGTGATGT AAATGATTGG TATAATGGGT
401 ATTATGGAAA AATATTACCC GGAGGAGATG TTATGGATTT TTCCAACTTT
451 TTTCAAAACC TCAGTACGTT AAAAATTGTA ACGAGTATCC TTGATTTACT
501 GATAGTTTGG TATGTACTTT ATCTTCTCAT CACGGTCTTT AAGGGAACTA
551 AAGCGATACA ATTACTTAAA GGGATATTAG TAATTGTTAT TGGTCAGCAG
601 ATAATTWTGA TATTGAACTT GACTGCMACA TCTAAATTAT YCRAWWYCGT
651 TATTCMATGG GGGGTATTAG CTTTAANAGT AATATTCCAA CCAGAAATTA
701 GACGTGCGTT AGAACAACTT GGTANAGGTA GCTTTTTAAA ACGCNATACT
751 TCTAATACGT ATAGTAAAGA TGAAGAGAAA TTGATTCAAT CGGTTTCAAA
801 GGCTGTGCAA TATATGGCTA AAAGACGTAT AGGTGCATTA ATTGTCTTTG
851 AAAAAGAAAC AGGTCTTCAA GATTATATTG AAACAGGTAT TGCCAATGGA
901 TTCAAATATT TCGCAAGAAC TTTTAATTAA TGTCTTTATA CCTAACACAC
951 CTTTACATGA TGGTGCAAKG ATTATTCAAG GCACGAARAT TGCAGCAGCA
1001 GCAAGTTATT TGCCATTGTC TGRWAGTCCT AAGATATCTA AAAGTTGGGT
1051 ACAAGACATA GAGCTGCGGT TGGTATTTCA GAAGTTATCT GATGCATTTA
1101 CCGTTATTGT ATCTGAAGAA ACTGGTGATA TTTCGGTAAC ATTTGATGGA
1151 AAATTACGAC GAGACATTTC AAACCGAAAT TTTTGAAGAA TTGCTTGCTG
1201 AACATTGGTT TGGCACACGC TTTCAAAAGA AAGKKKTGAA ATAATATGCT
1251 AGAAAKTAAA TGGGGCTTGA GATTTATTGC CTTTCTTTTT GGCATTGTTT
1301 TTCTTTTTAT CTGTTAACAA TGTTTTTGGA AATATTCTTT AAACACTGGT
1351 AATTCTTGGT CAAAAGTCTA GTAAAACGGA TTCAAGATGT ACCCGTTGAA
1401 ATTCTTTATA ACAACTAAAG ATTTGCATTT AACAAAAGCG CCTGAAACAG
1451 TTAATGTGAC TATTTCAGGA CCACAATCAA AGATAATAAA AATTGAAAAT
1501 CCAGAAGATT TAAGAGTAGT GATTGATTTA TCAAATGCTA AAGCTGGAAA
1551 ATATCAAGAA GAAGTATCAA GTTAAAGGGT TAGCTGATGA CATTCATTAT
1601 TCTGTAAAAC CTAAATTAGC AAATATTACG CTTGAAAACA AAGTAACTAA
1651 AAAGATGACA GTTCAACCTG ATGTAAGTCA GAGTGATATT GATCCACTTT
1701 ATAAAATTAC AAAGCAAGAA GTTTCACCAC AAACAGTTAA AGTAACAGGT
1751 GGAGAAGAAC AATTGAATGA TATCGCTTAT TTAAAAGCCA CTTTTAAAAC
1801 TAATAAAAAG ATTAATGGTG ACACAAAAGA TGTCGCAGAA GTAACGGCTT

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1851  TTGATAAAAA ACTGAATAAA TTAAATGTAT CGATTCAACC TAATGAAGTG
1901  AATTTACAAG TTAAAGTAGA GCCTTTTAGC AAAAAGGTTA AAGTAAATGT
1951  TAAACAGAAA GGTAGTTTRS CAGATGATAA AGAGTTAAGT TCGATTGATT
2001  TAGAAGATAA AGAAATTGAA TCTTCGGTAG TCGAGATGAC TTMCAAAATA
2051  TAAGCGAAGT TGATGCAGAA GTAGATTTAG ATGGTATTTC AGAATCAACT
2101  GAAAAGACTG TAAAAATCAA TTTACCAGAA CATGTCACTA AAGCACAACC
2151  AAGTGAAACG AAGGCTTATA TAAATGTAAA ATAAATAGCT AAATTAAAGG
2201  AGAGTAAACA ATGGGAAAAT ATTTTGGTAC AGACGGAGTA AGAGGTGTCG
2251  CAAACCAAGA ACTAACACCT GAATTGGCAT TTAAATTAGG AAGATACGGT
2301  GGCTATGTTC TAGCACATAA TAAAGGTGAA AAACACCCAC GTGTACTTGT
2351  AGGTCGCGAT ACTAGAGTTT CAGGTGAAAT GTTAGAATCA GCATTAATAG
2401  CTGGTTTGAT TTCAATTGGT GCAGAAGTGA TGCGATTAGG TATTATTTCA
2451  ACACCAGGTG TTGCATATTT AACACGCGAT ATGGGTGCAG AGTTAGGTGT
2501  AATGATTTCA GCCTCTCATA ATCCAGTTGC AGATAATGGT ATTAAATTCT
2551  TTGSCTCGAC CNCCNNGCTN GCA
```

Mutant: NT19

Phenotype: temperature sensitivity

Sequence map: Mutant NT19 is complemented by pMP49, which contains a 1.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 31. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the nucleic acid level to the *rnpA* gene, which encodes the catalytic RNA component RNAse P, from the bacilli *B. megaterium*, *B. subtilis*, and *B. stearothermophilus* as well as from other prokaryotes. The strongest similarity observed is to the *rnpA* Genbank entry from *B. subtilis* (Genbank Accession No.M13175; published in Reich, C.. *et al. J. Biol. Chem.*, 261 (1986) 7888-7893).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP49, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP49

SEQ ID NO. 18

pMP49 Length: 1962 nt

```
   1 GTGCTTCCAC CAATACGTTC CACCATATGG AGGATTTCCA ATTAACGCCA
  51 CCGGTTCTTC TGTATCAATT GTTAATGTAT TGACATCTTT TACACTAAAT
 101 TTAATAATAT CAGACAACCC AACTTCTTCA GCGTTACGCT TAGCAATCTC
 151 TACCATTTCT GGATCGATAT CAGAAGCATA TACTTCGATT TCTTTATCAT
 201 AATCAGCCAT CTTATCCGCT TCATCACGGT AATCATCATA AATATTTGCT
 251 GGCATGATGT TCCATTGCTC TGATACGAAC TCGCGATTAA AACCAGGTGC
 301 GATATTTTGA GCAATTAAAC AAGCTTCTAT AGCTATTGTA CCCGAACCGC
 351 AAAATGGATC AATTAAAGGT GTATCACCTT TCCAGTTTGC AAGACGGATT
 401 AAACTTGCTG CCAACGTTTC TTTAATTGGT GCTTCACCTT GTGCTAATCT
 451 ATAACCACGT CTGTTCAAAC CAGAACCTGA TGTGTCGATA GTCAATAATA
 501 CATTATCTTT TAAAATGGCA ACTTCAACAG GGTATTTGGC ACCTGATTCA
 551 TTTAACCAAC CTTTTTCGTT ATATGCGCGA CGTAATCGTT CAACAATAGC
 601 TTTCTTAGTT ATCGCCTGAC AATCTGGCAC ACTATGTAGT GTTGATTTAA
 651 CGCTTCTACC TTGAACTGGG AAGTTACCCT CTTTATCAAT TATAGATTCC
 701 CAAGGGAGCG CTTTGGTTTG TTCGAATAAT TCGTCAAACG TTGTTGCGTW
 751 AAAACGTCCA ACAACAATTT TGATTCGGTC TGCTGTGCGC AACCATAAAT
 801 TTGCCTTTAC AATTGCACTT GCGTCTCCTT CAAAAAATAT ACGACCATTT
 851 TCAACATTTG TTTCATAGCC TAATTCTTGA ATTTCCCTAG CAACAACAGC
 901 TTCTAATCCC ATCGGACAAA CTGCAAGTAA TTGAAACATA TATGATTCTC
 951 CTTTTATACA GGTATTTTAT TCTTAGCTTG TGTTTTTTAT ACATTTCCAA
1001 CAAATTTAAT CGCTGATACA TTAACGCATC CGCTTACTAT TTTAAAACAA
1051 GGCAGTGTCA TTATATCAAG ACAAGGCGTT AATTTTAAGT GTCTTCTTTY
1101 CATGAAAAAA GCTCTCCMTC ATCTAGGAGA GCTAAACTAG TAGTGATATT
1151 TCTATAAGCC ATGTTCTGTT CCATCGTACT CATCACGTGC ACTAGTCACA
1201 CTGGTACTCA GGTGATAACC ATCTGTCTAC ACCACTTCAT TTCGCGAAGT
1251 GTGTYTCGTT TATACGTTGA ATTCCGTTAA ACAAGTGCTC CTACCAAATT
1301 TGGATTGCTC AACTCGAGGG GTTTACCGCG TTCCACCTTT TATATTTCTA
1351 TAAAAGCTAA CGTCACTGTG GCACTTTCAA ATTACTCTAT CCATATCGAA
1401 AGACTTAGGA TATTTCATTG CCGTCAAATT AATGCCTTGA TTTATTGTTT
1451 CAYCAAGCRC GAACACTACA ATCATCTCAG ACTGTGTGAG CATGGACTTT
1501 CCTCTATATA ATATAGCGAT TACCCAAAAT ATCACTTTTA AAATTATAAC
1551 ATAGTCATTA TTAGTAAGAC AGTTAAACTT TTGTATTTAG TAATTATTTA
1601 CCAAATACAG CTTTTTCTAA GTTTGAAATA CGTTTTAAAA TATCTACATT
1651 ATTTGAAGAT GTATTTGTTG TTGTATTATT CGAAGAAAAA CTTTTATTGT
1701 CCTGAGGTCT TGATGTTGCT ACACGTAGTC TTAATTCTTC TAATTCTTTT
1751 TTAAGTTTAT GATTCTCTTC TGATAATTTT ACAACTTCAT TATTCATATC
1801 GGCCATTTTT TGATAATCAG CAATAATGTC ATCTAAAAAT GCATCTACTT
1851 CTTCTCTTCT ATAGCCACGA GCCATCGTTT TTTCAAAATC TTTTTCATAA
1901 ATATCTTTTG CTGATAATTT CAATGAAACA TCTGACATTT TTTCCACCTC
1951 ATTAGAAACT TT
```

Mutant: NT23

Phenotype: temperature sensitivity

Sequence map: Mutant NT23 is complemented by pMP55, which contains a 5.2 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 32. Database searches at both the nucleic acid and peptide levels reveal

limited similarity at the protein level only to *S. aureus* proteins FemA and FemB, suggesting that clone pMP55 contains a new Fem-like protein. Since the Fem proteins are involved in peptidoglycan formation, this new Fem-like protein is likely to make an attractive candidate for screening antibacterial agents. Since clone pMP55 does not map to the same location as the *femAB* locus (data not shown here), the protein is neither FemA nor FemB and represents a novel gene.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP55, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP55, a 5000 bp genomic fragment

SEQ ID NO. 19

```
pMP55  Length: 5253 nt

   1  TAACTGGACT ACWACCGCCA ACTRAGTATT GAATTGTTTT AACATGCTTT
  51  TCCTGTTTTA AATATTTTTA AACATCTTTC GCATGATTCA ACACTGCTTG
 101  CTCCGTTTCA CCAGGCTTCG GTGTATAAGT AATAGCTAAA AATTTATCGT
 151  CACCTGCTGA AATAAAGCTA GTGCCTAGTC TCGGTCCTCC AAATACAATA
 201  GTTGCAACCA AAATTAATGT ACTTAATATA ATTWCAATCC ACTTATGATT
 251  TAATGACCAA TGTAATACTT TTTTATAAGT TGTACTAACA ACACCTAATC
 301  CTTCTTGATG TTGTTTATTA CGACGTTTAA CGCCTTTTTT AAATAGTGTA
 351  GCTGCCAACG CTGGAACGAG TGTAATTGAC ACTAATAACG ATGCTAATAA
 401  ACTAAATGCA ATAGCCAATG CAAAAGGTCT AAACATTTCG CCTACTGAAC
 451  CTGATACAAA CACAAGTGGT AAGAAGACGA TAATAGKAAC TAGTGTCGAT
 501  GRCATTATTG GTTTAAATAC TTCAGTTGTC GCACTGATAA TTAAATTTTC
 551  ACCTTTTAGT TGGTTCTTCT GAATCTGTTA AGCGTCGATA AATATTTTCA
 601  MCAACTACAA TCGAATCGTC TATCACACGT CCAATCGCTA CTGTTAATGC
 651  ACCTAACGTT AGTATATTCA ATGAMACATC ACTCAATTTC AGAGCAATAA
 701  GCGSCATAAG AAGTGATAAC GGMATCGATA TMATAGAAAT TGCCGTCGTA
 751  CGAATGTTTC TTAAAAACAG CAAAATAACT ATAATTGCCA CGRATTGTAC
 801  CTAATGATGC TTTTTCAACC ATCGTATAAA GTGATTTCTC AACAGGCTTT
 851  GCAGTATCCA TTGTTTTTGT GACATTAAAA TCTTTATTTT CATCAACGAA
 901  TGTATCAATT TTACGTTGTA CATCTTTGGC TACTTGAACT GTATTGGCAT
 951  CTTGAGCTTT AGTTATTTGT AGATTAACCG CATCCTTTCC ATTCGTTTTA
1001  GAAATAGAAG TACGCACATC ACCAACTGTA ATATCAGCTA AATCTCCTAG
1051  TTTCGCTGTC GGCATACCAC TTATATTATT TGGTGCTGAC GCTTTTGAAT
1101  TTTGCTGTGG TGATGCCTGA TTAACGTCTG ACATGGCTGA AATTTTGTTT
1151  ATTGTCACTT TGGGATTGAG ATTGCCCTTG TCCTCCTGCC AACGTTAATG
```

1201 GAATATTTAT GTTTTTAAAA GCATCAACAG ATTGATATTG ACCATCAACA
1251 ACAATTGATT TATCTTTATC ACCAAATTGG AACAATCCAA GTGGCGTTGT
1301 TCTTGTTGCC GTTTTTAGAT AGTTTTCTAC ATCATCAGCA GTCAACCCAT
1351 ATTTTCAAGT TCATTTTGCT TAAATTTAAG GGTGATTTCA CGGTTCGTCT
1401 GCCCATTTAA TTGCGCATTT TGNACACCAT CTACCGTTTG CAATTTTGGT
1451 ATNAATTGTT CATTCAGTAC TTTCGTTACT TTTTTCAAGT CATTCNCTTT
1501 ATTTGAAAAT GAATATGCTA AAACCGGAAA AGCATCCATC GAATTACGTC
1551 NTANTTCTGG TTGACCAACT TCATCTTTAA ATTTAATTTT NTNTATTTCT
1601 NTTNTAAGCT GTTCTTCTGC TTTATCCAAA TCTGTATTMT TTTCATATTC
1651 AACTGTTACA ATTGAAGCAT TTTGTATGGA TTGCGTTTTA ACATTTTTCA
1701 CATATGCCAA TGATCTTACY TGAWTGTCAA TTTTACTACT TATTTCATCT
1751 TGGGTACTTT GTGGCGTTGC ACCCGGCATT GTTGTTGTAA CTGAAATAAC
1801 TGGATKTTGT ACATTTGGTA KTAATTCTMA TTTCAATTTA GCACTCGCAT
1851 ATACACCGCC CAAGACAACT WAAACAACCA TTAMAAAGAT AGCAAACYTA
1901 TTCCCTAAAA RGAAAATTGT AATAGCTTTT TTAWCAACAG TMCTYCCCCC
1951 TCTTTCACTA WAATTCAAAA AATTATTTTA CTCAACCATY CTAWWWTGTG
2001 TAAAAAAAAT CTGAACGCAA ATGACAGYCT TATGAGCGTT CAGATTTCAG
2051 YCGTTAATCT ATTTYCGTTT TAATTTACGA GATATTTTAA TTTTAGCTTT
2101 TGTTAAACGC GGTTTAACTT GCTCAATTAA TTGGYACAAT GGCTGATTCA
2151 ATACATAATC AAATTCACCA ATCTTTTCAC TTAAGTATGT TCCCCACACT
2201 TTTTTAAATG CCCATAATCC ATAATGTTCT GAGTCTTTAT CTGGATCATT
2251 ATCTGTACCA CCGAAATCGT AAGTTGTTGC ACCATGTTCA CGTGCATACT
2301 TCATCATCGT ATACTGCATA TGATGATTTG GTAAAAAATC TCTAAATTCA
2351 TTAGAAGACG CACCATATAA GTAATATGAT TTTGAGCCAG CAAACATTAA
2401 TAGTGCACCA GAAAGATAAA TACCTTCAGG ATGTTCCTTT TCTAAAGCTT
2451 CTAGGTCTCG TTTTAAATCT TCATTTTTAG CAATTTTATT TTGCGCATCA
2501 TTAATCATAT TTTGCGCTTT TTTAGCTTGC TTTTCAGATG TTTTCATCTT
2551 CTGCTGCCAT TTAGCAATTT CGGCATGAAG TTCATTCAAT TCTTGATTTA
2601 CTTTCGCTAT ATTTTCTTTT GGATCCAACT TTACTAAAAA TAGTTCAGCA
2651 TCTCCATCTT CATGCAACGC ATCATAAATA TTTTCAAAGT AACTAATATC
2701 ACGCGTTAAG AAGCCATCGC GTTCCCCAGT GATTTTCATT AACTCAGCAA
2751 ATGTTTTTAA ACCTTCTCTA TCAGATCGTT CTACTGTCGT ACCTCGCTTT
2801 AAAGCCAAGC GCACTTTTGA ACGATTTCGG CGTTCAAAAC TATTTAATAA
2851 CTCATCATCA TTTTTATCAA TTGGTGTAAT CATAGTCATA CGTGGTTGGA
2901 TGTAGTCTTT TGATAAACCT TCTTTAAATC CTTTATGTTT AAAACCAAGC
2951 GCTTTCAAAT TTTGCAAAGC ATCTGTRCCT TTATCAACTT CAACATCAGG
3001 ATCGRTTTTA ATTGCATACG CTTTCTCAGC TTTAGCAATT TCTTTTGCAC
3051 TGTCTAACMA TGSMTTTAAC GYTTCTTTAT TACTATTAAT CAACAACCAA
3101 AACCMCGCGR RAWTATWACM TAGSGTATAA GGTAATTTAG GTACTTTTTT
3151 AAAAAGTAAC TGCGCAACAC CCTGGAACTT SMCCGTCACG ACCTACAGCG
3201 ATTCTTCGCG CGTACCATCC AGTTAATTTC TTTGTTTCTG CCCATTTCGT
3251 TAATTGTAAT AAATCTCCAT TTGGGTGGGR WTTWACAAAT GCGTCATGTT
3301 CCTGATTAGG KGATATGCAT CTTTTCCATG ATTTATGATA TCTCCTTCTA
3351 TTTAACAATA CCTTTAATTA TACAGTTTGT ATCTTATAGT GTCGATTCAG
3401 AGCTTGTGTA AGATTTGAAC TCTTATTTTT GGAAATGTCC ATGCTCCAAT
3451 TAATAGTTTA GCAAGTTCAA ATTTACCCAT TTTAATTGTG AATCATTTTA
3501 TATCTATGTT TCGTGTTAAA TTTAATGTTA TCGTACARTT AATACTTTTC
3551 AACTAGTTAC CTATACTTCA ATATACTTTC ATCATCTAAC ACGATATTCA
3601 TTTCTAARAA TGAACCAACT TGACTTCAAT GAATAAATTT TTCCTCAAGC
3651 AACCACATTA ATGTTCATAT ACAATTACCC CTGTTATAAT GTCAATAATC
3701 TAACAATGAG GTGTTTGATA TGAGAACAAT TATTTTAAGT CTATTTATAA

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3751  TTATGRACAT CGTTGCAATC ATTATGACAT TGAGTCAACC TCTCCACCGT
3801  GAATTACTTT AGTTTACGGG TTATACTTAT CTTTTTCACA TTTATATTAT
3851  CAATCTTTTT CATTTTAATT AAGTCATCAC GATTAAATAA TATATTAACG
3901  ATTMWWTCCA TTGTGCTTGT CATTATTCAT ATGGGCATTC TCGCTCATAG
3951  CACTTACGTA TATTTATACT AATGGTTCAA AGCGATAAAT AGCACCTCTG
4001  ATAAAAATTG AATATGGTGA AGTTGCTTGT GCGTCTTTTA TGATAACCGA
4051  ATGATATTTT GAAACTTTAC CATCTTCAAT TCTAAAATAA ATATCATCAT
4101  TTTTTAAAAT CAAATCTGTG TAATGGTCAT TTYKTCHACA ATGTCCATAT
4151  CAARCCATTT CAACCAATTC GATACTGTWK GTGATCGGTT TTTACTTTTC
4201  ACAATAACAG TTTCAAWTGA AAATTGTTTT TGAAAATATT TTTGCAATTT
4251  TTTAGTACGC ATGGAATCAC TTTCTTCCCA TTGAATAAAA AATGGTGGCT
4301  TAATTTCATC ATCATCCTGA TTCATTATAT AAAGCAATTG CCACTTTACC
4351  TWCACCATCT TTATGTGTAT CTCTTTCCAT TTGAATCGGC CCTACTACTT
4401  CAACCTGCTC ACTNTGTAGT TTATTTTTAA CTGCCTCTAT ATCATTTGTA
4451  CGCAAACAAA TATTTATTAA AGCCTTGCTC ATACTTCTCT TGAACAATTT
4501  GAGTAGCAAA AGCGACTCCG CCTTCTATCG TTTTTGCCAT CTTTTTCAAC
4551  TTTTCATTAT TTTACTACAT CTAGTAGCTC AAGATAATTT CATTGATATW
4601  ACCTAAKKTA TTGAATGTTC CATATTTATG ATGATACCCA CCTGAATGTA
4651  ATTTTATAAC ATCCTCCTGG AAAACTAAAC CGATCTAACT GATCTATATA
4701  ATGAATGATG TGATCANATT TCAATATCAT TAGTATCCCC CTATTTACAT
4751  GTAATTACGC TTATTTTAAA CAAAGTAWAA TTATTTTTGC YCTTAATAAT
4801  TATATAKTGA YYYCWAATTG CTCCCGTTTT ATAATTACTA TTGTTGTAAA
4851  ARGGTTAGCT AAGCTAACTA TTTTGCCTTA GGAGATGTCA CTATGCTATC
4901  ACAAGAATTT TTCAATAGTT TTATAACAAT ATAYCGCCCC TATTTAAAAT
4951  TAGCCGAGCC GATTTTAGRA AAACACAATA TATATTATGG CCAATGGTTA
5001  ATCTTACGCG ATATCGCTAA ACATCAGCCC ACTACTCTCA TTGNAATTTC
5051  ACATAGACGG GCAATTGAAA AGCCTACTGC AAGAAAAACT TTAAAAGCTC
5101  TAATAGGAAA TGACCTTATW ACAGTAGAAA ACAGNTTAGA GGATAAACNA
5151  CAAAAGNTTT TAACTTTAAC ACCTAAAGGG CATKAATTAT ATGAGATTGT
5201  TTGTCTTGAT GNACAAAAGC TCCNACAAGC AGNNAGTTGC CAAAACAAAG
5251  ATT
```

Mutant: NT27

Phenotype: temperature sensitivity

Sequence map: Mutant NT27 is complemented by pMP59, which contains a 3.2 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 33. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to two hypothetical ORFs from *B. subtilis*. These hypothetical ORFs are also found in other bacteria, but in all cases, nothing has been reported in the literature about the functions of the corresponding gene products.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP59, starting with the

standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP59

SEQ ID NO. 20

pMP59 Length: 3263 nt

```
   1 ACATTGAMAA AGATCACCCA TTACAACCAC ATACAGATGC AGTAGAAGTT
  51 TAAAACACAT TTTTCTAATT ATCAAAGCTT AGGATAAATA TGATGTCCTA
 101 AGCTTTTCCT TTTACAACTT TTTCGAATAA ACAACAGTTA AATATATTCA
 151 CCTTTCTACC AAACTTTTTA TCCCCTCATT TAAATTTTAC CGGKYTCATA
 201 TAAAATCCTT TAATTCTTTC TTAACATTAW TTTWTWATCT CTACATYTAT
 251 TTTAATAAAT AGAACTGCAC ATTTATTCGA AATACTTAGA TTTCTAGTGA
 301 GATAAACTGC TTTATTTATT ATCATTCATC ATGTAAAATA AGATTTAACT
 351 GAAATTTTAG TGTTATTTCA CTAATTTTTT AAAATGAACG ACATGATGAA
 401 CCTAGTTATT AACCAAATCG TTATTAAGTT ACATTATAGA GATGATTGGA
 451 ATGAATTTAT CGATATATAC TCCAATACGA TTTTACTAGG GTTAACAATA
 501 AATTAAACAA ACATTCTTAG GAGGRATTTT TAACATGGCA GTATTTAAAG
 551 TTTTTTATCA ACATAACAGA GTACGAGGTR RTTGTGCGTG AAAATACACA
 601 ATCACTTTAT GTTGAAGCTC ARACAGAAGA ACAAGTAGCG TCGTTACTTG
 651 AAAGATCGTA ATTTTAATAT CGAATTTATC ACTAAATTAG AGGGCGCACA
 701 TTTAGATTAC GAAAAAGAAA ACTCAGCAAC ACTTTAATGT GGAGATTGCT
 751 AAATAATGAA ACAATTACAT CCAAATGAAG TAGGTGTATA TGCACTTGGA
 801 GGTCTAGGTG AAATCGGTAA AAATACTTAT GCAGTTGAGT ATAAAGACGA
 851 AATTGTCATT ATCGATGCCG GTATCAAATT CCCTGATGAT AACTTATTAG
 901 GGATTGATTA TGTTATACCT GACTACACAT ATCTAGTTCA AAACCAAGAT
 951 AAAATTGTTG GCCTATTTAT AACACATGGT CACGAAGACC ATATAGGCGG
1001 TGTGCCCTTC CTATTAAAAC AACTTAATAT ACCTATTTAT GGTGGTCCTT
1051 TAGCATTAGG TTTAATCCGT AATAAACTTG AAGAAACATC ATTTATTACG
1101 TACTGCTAAA CTAAATGAAA TCAATGAGGA CAGTGTGATT AAATCTAAGC
1151 ACTTTACGAT TTCTTTCTAC TTAACTACAC ATAGTATTCC TGAAACTTAT
1201 GGCGTCATCG TAGATACACC TGAAGGAAAA KTAGTTCATA CCGGTGACTT
1251 TAAATTTGAT TTTACACCTG TAGGCAAACC AGCAAACATT GCTAAAATGG
1301 CTCAATTAGG CGAAGAAGGC GTTCTATGTT TACTTTCAGA CTCAACAAAT
1351 TCACTTGTGC CTGATTTTAC TTTAAGCGAA CGTTGAAGTT GGTCAAAACG
1401 TTAGATAAGA TCTTCCGTAA TTGTAAAGGT CCGTATTATA TTTGCTACCT
1451 TCGCTTCTAA TATTTACCGA GTTCAACAAG CAGTTGAAGC TGCTATCAAA
1501 AATAACCGTA AAATTGTTAC KTTCGGTCCG TTCGATGGAA AACAATATTA
1551 AAATAGKTAT GGAACTTGGT TATATTAAAG CACCACCTGA AACATTTATT
1601 GAACCTAATA AAATTAATAC CGTACCGAAG CATGAGTTAT TGATACTATG
1651 TACTGGTTCA CAAGGTGAAC CAATGGCAGC ATTATCTAGA ATTGCTAATG
1701 GTACTCATAA GCAAATTAAA ATTATACCTG AAGATACCGT TGTATTTAGT
1751 TCATCACCTA TCCCAGGTAA TACAAAAAGT TATTAACAGA ACTATTAATT
1801 CCTTGTATAA AGCTGGTGCA GATGTTATCC ATAGCAAGAT TTCTAACATC
1851 CATACTTCAG GGCATGGTTC TCAAGGGTGA TCAACAATTA ATGCTTCCGA
1901 TTAATCAAGC CGAAATATTT CTTACCTATT CATGGTGAAT ACCGTATGTT
1951 AAAAGCACAT GGTGAGACTG GTGTTGAATG CGSSKTTGAA GAAGATAATG
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2001  TCTTCATCTT TGATATTGGA GATGTCTTAG CTTTAACACM CGATTCAGCA
2051  CGTAAAGCTG KTCGCATTCC ATCTGGTAAT GWACTTGTTG ATGGTAGTGG
2101  TATCGGTGAT ATCGGTAATG TTGTAATAAG AGACCGTAAG CTATTATCTG
2151  AAGAAGGTTT AGTTATCGTT GTTGTTAGTA TTGATTTTAA TACAAATAAA
2201  TTACTTTCTG GTCCAGACAT TATTTCTCGA GGATTTGTAT ATATGAGGGA
2251  ATCAGGTCAA TTAATTTATG ATGCACAACG CMAAAWCMAA ACTGATGTTT
2301  ATTAGTWAGT TWAATCCAAA ATAAAGAWAT TCAATGGCAT CAGATTAAAT
2351  CTTCTATCAT TGAAACATTA CAACCTTATT TATTKGAAAA AACAGCTAGR
2401  AAACCAATGA TTTTACCAGT CATTATGGAA GGTAAACGAA CAAAARGAAT
2451  CAAACAATAA ATAATCAAAA AGCTACTAAC TTTGAAGTGA AGTTTTAATT
2501  AAACTCACCC ACCCATTGTT AGTAGCTTTT TCTTTATATA TGATGAGCTT
2551  GAGACATAAA TCAATGTTCA ATGCTCTACA AAGTTATATT GGCAGTAGTT
2601  GACTGAACGA AAATGCGCTT GTWACAWGCT TTTTTCAATT STASTCAGGG
2651  GCCCCWACAT AGAGAATTTC GAAAAGAAAT TCTACAGGCA ATGCGAGTTG
2701  GGGTGTGGGC CCCAACAAAG AGAAATTGGA TTCCCCAATT TCTACAGACA
2751  ATGTAAGTTG GGGTGGGACG ACGGAAATAA ATTTTGAGAA AATATCATTT
2801  CTGTCCCCAC TCCCGATTAT CTCGTCGCAA TATTTTTTTC AAAGCGATTT
2851  AAATCATTAT CCATGTCCCA ATCATGATTA AAATATCACC TATTTCTAAA
2901  TTAATATTTG GATTTGGTGA AATGATGAAC TCTTTGCCTC GTTTAATTGC
2951  AATAATGTTA ATTCCATATT GTGCTCTTAT ATCTAAATCA ATGATAGACT
3001  GCCCCGCCAT CTTTTCAGTT GCTTTCAATT CTACAATAGA ATGCTCGTCT
3051  GCCAACTCAA GATAATCAAG TACACTTGCA CTCGCAACAT TATGCGCNAT
3101  ACGTCTACCC ATATCACGCT CAGGGTGCAC AACCGTATCT GCTCCAATTT
3151  TATTTAAAAT CTTTGCNTGA TAATCATTTT GTGCTCTTAG CAGTTACTTT
3201  TTTTACACCT AACTCTTTTA AAATTAAAGT CGTCAACGTA CTTGNTTGAA
3251  TATTTTCACC AAT
```

Mutant: NT28

Phenotype: temperature sensitivity

Sequence map: Mutant NT28 is complemented by pMP60, which contains a 4.7 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 34, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity of clone pMP60 at both the nucleic acid and peptide levels to the *polC* gene, encoding DNA Polymerase III alpha subunit, from *S. aureus*(Genbank Accession No. Z48003; unpublished as of 1995). The relative size and orientation of the complete ORF encoding Pol III is depicted by an arrow in the map.

DNA sequence data: The following DNA sequence data was generated by using the standard sequencing primers SP6 and

T7, and can be used to demonstrate identity between clone pMP60 and Genbank entry Z48003:

subclone 1022, a 900 bp EcoR I fragment

SEQ ID NO. 21

```
1022.sp6  Length: 510 nt
  1  GGGTACCGAG CTCGAATTCG AGGTGTACGG TAGAAATACT TCACCAATGA
 51  TGCACTTACA ATTTTAAATA GATTTTNAAG ACCTTGTTGG TTTTGTACAA
101  TTAATGTGAC ATGACTAGGT CTTGCACGTT TATATGCATC TNCATTACTG
151  AGTTTTTTGT TGATTTCGTT ATGATTTAAT ACGCCTAATT CTTTCATTTG
201  TTGAACCATT TTNATGAAAA TGTAAGCTGT TGCTTCTGTA TCATAAATGG
251  CACGGTGATG TTGCGTTAAT TCTACGCCAT ATTTTTTAGC CAAGAAATTC
301  AAACCATGTT TACCATATTC AGTATTAATC GTACGNGATA ATTCTAAAGT
351  ATCGNTAACA CCATTCGTTG ATGGTCCAAA CCCAAGACGT TCATATCCCG
401  TATCGATGNN GCCCATATCA AACGGAGCAT TATGCGTTAC GGTTTTCGNA
451  TCGGCAACCC TTCTTAAACT CTGTAAGNAC TTCTTCATTT CAGGGGATCT
501  NCTANCATAT
```

subclone 1023, a 1200 bp EcoR I fragment

SEQ ID NO. 22

```
1023.sp6  Length: 278 nt
  1  GGGTACCGAG CTCGAATTCT ACACGCTTTT CTTCAGCCTT ATCTTTTTTT
 51  GTCGCTTTTT TAATCTCTTC AATATCAGAC ATCATCATAA CTAAATCTCT
101  AATAAATGTA TCTCCTTCAA TACGNCCTTG AGCCCTAACC CATTTACCAA
151  CANTTAGNGC TTTAAAATGT TCTAAATCAT CTTTGTTTTT ACGAGTAAAC
201  ATTTTTAAAA CTAAAGNGTC CGTATAGTCA GTCACTTTAA TTTCTACGGT
251  ATGGNGGCCA CTTTTAAGTT CTTTTAAG
```

subclone 1024, a 1400 bp EcoR I fragment

SEQ ID NO. 23

```
1024.sp6  Length: 400 nt
  1  GGGTACCGAG CTCGAATTCT GGTACCCCAA ATGTACCTGT TTTACATAAA
 51  ATTTCATCTT CAGTAACACC CAAACTTTCA GGTGTACTAA ATATCTGCAT
101  AACTNCTTTA TCATCTACAG GTATTGTTTT TGGNTCAATT CCTGATAAAT
151  CTTGAAGCAT ACGAATCATT GTTGGNTCAT CGTGTCCAAG TATATCANGT
201  TTTAATACAT TATCATGAAT AGAATGGAAA TCAAAATGTG TCGTCATCCA
251  TGCTGAATTT TGATCATCGG CAGGATATTG TATCGGCGTA AAATCATAAA
301  TATCCATGTA ATCAGGTACT ACAATAATAC CCCCTGGNTG CTGTCCAGTT
351  GTACGTTTAA CACCTGTACA TCCTTTAACG NGTCGATCTA TTTCAGCACC
```

subclone 1025, a 1200 bp EcoR I/ Hind III fragment

SEQ ID NO. 24

```
1025.sp6  Length: 528 nt
```

```
  1  GATCATTTGC ATCCATAGCT TCACTTATTT NTCCAGAAGC TAGCGTACAA
 51  TCATTTAAAT CTACGCCACC TTCTTTATCA ATAGAGATTC TAAGAAAATN
101  ATCTCTACCC TCTTTGACAT ATTCAACGTC TACAAGTTCA AAATTCAAGT
151  CTTCCATAAT TGGTTTAACA ATCACTTCTA CTTGTCCTGT AATTTTNCTC
201  ATACAGGCCT CCCTTTTTGG CAAATAGAAA AGAGCGGGAA TCTCCCACTC
251  TTCTGCCTGA GTTCACTAAT TTTTAAGCAA CTTAATTATA GCATAAGTTT
301  ATGCTTGAAA CAAATGACTT CACTATTAAT CAGAGATTCT TGTAAAAGTT
351  TGTCCCTTTA TTTCACCATT ACATTTGAAT NGNCTCGTNA GNCATTGTAA
401  AGAGATNCGG GCATAATTTT GTGTCCAGCA TCAATTTTGG TATTTCTTGT
451  CTTACGGCTT ACGGTTNATT AAATACCTNG GNTTTTTNTC TTTTACCTNT
501  NATATNTCGN ANGNTGGGNT TTTTCNNG
```

Mutant: NT29

Phenotype: temperature sensitivity

Sequence map: Mutant NT29 is complemented by pMP62, which contains a 5.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 35, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity between clone pMP62 and the *gyrBA* locus of *S. aureus* (Genbank Accession No. M86227; published in Margerrison, E.E., *et al. J. Bacteriology*, 174 (1992) 1596-1603), which encodes DNA gyrase (EC 5.99.1.3). Arrows above the restriction map indicate relative size and position of the ORFs, demonstrating that both *gyrB* and *gyrA* genes are fully contained within clone pMP62 and are likely to be expressed.

DNA sequence data: The following DNA sequence data are those obtained from subclones of clone pMP62, using standard sequencing conditions and the primers T7 or SP6. These data can be used to demonstrate identity between the pMP62 clone and Genbank entry M86227.

subclone 29.2e.a, a 550 bp EcoR I fragment
SEQ ID NO. 25

29.2e.a.sp6 LENGTH: 557 nt

```
  1  CAGCCGACAG TTNACAACCA GCNTCACCGT NAGACAGCAA ACGCCACAAA
 51  CTACAAGGNT CCAAATGNCT AGACAATACT GGTGNAAGGC ANGTAATAAT
101  ACGACATTAA CATTTGATGA TCCTGCCATA TCAACAGNTC AGAATAGACA
151  GGATCCAACT GTAACTGTTA CAGATAAAGT AAATGGTTAT TCATTAATTA
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201 ACAACGGTAA GATTGGTTTC GTTAACTCAG AATTAAGACG AAGCGATATG
251 TTTGATAAGA ATAACCCTCA AAACTATCAA GCTAAAGGAA ACGTGGCTGC
301 ATTAGGTCGT GTGAATGCAA ATGATTCTAC AGATCATGGT AACTTTAACG
351 GTATTTCAAA AACTGTAAAT GTAAAACCAG NTTCAGAATT AATTATTAAC
401 TTTACTACTA TGCAAACCGG ATAGTNAGCA AGGTGCAACA AATTTAGTTA
451 TTAAAGGATG CTAAGGAANN TACTGNNTTA GCACCTGTAA AATGTTGCTT
501 AGGCTGGTCC TGCACATTTA TTTTAAGGTC CNNCTTGTNC TGNTNGGCTC
551 TNGGGGG

SEQ ID NO. 26

29.2e.a.t7 LENGTH: 527 nt

1 GTCGATCAGC ATCATTGGTA CTTTAAATAA ATGTGCAGTA CCAGTCTTAG
51 CAACATTTAC AGTTGCTAAT TCAGTATTTT CNTTAGCATC TTTAATAACT
101 AANTTTNTNG CACCTTGCNT ACTATTCGTT TGCATAGTAG TAAAGTTAAT
151 AATTAATTCT GANTCTGGTT TTACATTTAC AGTTTTTGAA ATACCGTTAA
201 AGTTACCATG ANCTGTAGNA TCATTTGCNT TCACACGGCC TAATGCAGCC
251 NCGGTTCCTT TAGCTTGATA GTTTTGAGGG GTATTCTTAT CAAACATATC
301 GNTTCGGCTT AATTCTGAGG TAACTGGNAC CNATCTTTAC CNTTGTTAAT
351 TAATGGNTTC CCCTTTACNT TAATCTGTAA CAGTTACAGT TGGGTCCCCG
401 TCTATTCTCA TCTGTTGGTA TGGCAGGGTC ACCACAATGN TAATGTCGGT
451 TTATACTGGN NTCNCCCGNA TTGCTTAGGT TTGGNGCTTG NGGTGTGCGN
501 TTNCTNGCTT CAGGGGNCTG CTGGGTT

subclone 29.2h.2a, a 1800 bp Hind III fragment

SEQ ID NO. 27

29.2h.2a.sp6 LENGTH: 578 nt

1 TGTGAGCTCC CATNACCACC AGTGCGNNCA TTGCCTGGGC TACCGATTGT
51 CAATTTAAAG TCTTCATCTT TAAAGAAAAT TTCAGTACCA TGTTTTTTAA
101 GTACAACAGT TGCACCTAAA CGATCAACTG CTTCACGATT ACGCTCATAT
151 GTCTGTTCCT CAATAGGAAT ACCACTTAAT CGTTCCCATT CTTTGAGGTG
201 TGGTGTAAAG ATCACACGAC ATGTAGGTAA TTGCGGTTTC AGTTTACTAA
251 AGATTGTAAT CGCATCGCCG TCTACGATTA AATTTTGATG CGGTTGTATA
301 TTTTGTAGTA GGAATGTAAT GGCATTATTT CCTTTGAAAT CAACGCCAAG
351 ACCTGGACCA ATTAGTATAC TGTCAGTCAT TTCAATCATT TTCGTCAACA
401 TTTTCGTATC ATTAATATCA ATAACCATCG CTTCTGGGCA ACGAGAATGT
451 AATGCTGAAT GATTTGTTGG ATGTGTAGTA CAGTGATTAA ACCACTACCG
501 CTAAATACAC ATGCACCGAG CCGCTAACAT AATGGCACCA CCTAAGTTAG
551 CAGATCGGCC CTCAGGATGA AGTTGCAT

SEQ ID NO. 28

29.2h.2a.t7 LENGTH: 534 nt

1 CGAGCCAGCA GNTTGCAGCG GCGTGTCCCA TAACTAAGGT GGTGCCATTA
51 TGTNAGCGGC TCGTCCATGT NTATTTGGCG GTAGTGGTTT AATCACTGTA
101 GCTACACATC CAACAAATCA TTCAGCATTA CATTCTCGTN GCCCAGAAGC
151 GATGGTTATT GATATTAATG ATACGAAAAT NTTGACGAAA ATNATTGAAA
201 TGACTGACAG TATACTAATN GGNCCAGGTC TTGGCGTTGA TTTCAAAGGA
251 AATAATGCCA TTNCATTCCT ACTACAAAAT ATACAACCGC ATCAAAATTT
301 AANCGTAGAC GGCGNTGCGA TTNCAATCTT TNGTAAACTG NAACCGCAAT

351 TACCTACATG TNGTGTGNNC TTNACACCAC ACCTCAAAGG NNTGGGNCGG
401 TTANGTGGTA TTCCNNTTGN GGACAGGCAT ATGGNGCGTA ATCGTGNAGC
451 AGTTGNTCGT TTAGGNGCAC TNTNGTCCTT AAAAAACATG GTCTGNATNT
501 CCTTTAANGN NGNNGCTTTA AATTGGCAAT CGGT

subclone 29.2he, 2400 bp Hind III, EcoR I fragment
SEQ ID NO. 29

29.2he.1.sp6 LENGTH: 565 nt
1 ACCATTCACA GTGNCATGCA TCATTGCACA CCAAATGNTG TTTGAAGAGG
51 TGTTTGTTTG TATAAGTTAT TTAAAATGAC ACTAGNCATT TGCATCCTTA
101 CGCACATCAA TAACGACACG CACACCAGTA CGTAAACTTG TTTCATCACG
151 TAAATCAGTG ATACCGTCAA TTTTCTTGTC ACGAACGAGC TCTGCAATTT
201 TTTCAATCAT ACGAGCCTTA TTCACTTGGA AAGGAATTTC AGTGACAACA
251 ATACGTTGAC GTCCGCCTCC ACGTTCTTCA ATAACTGCAC GAGAACGCAT
301 TTGAATTGAA CCACGNCCTG TTTCATATGC ACGTCTAATA CCACTCTTAC
351 CTAAAATAAG TCCNGCAGTT GGGGAATCAG GACCTTCAAT ATCCTCCATT
401 AACTCAGCAA ATTGNAATNT CAAGGGGTCT TTACTTTAAG GCTNAGNNCA
451 CCCTTGGTTA ATTCTGTTAA GTTATTGTGG TGGGATATTT CGGTTGCCAT
501 NCCTNCCNCG GGTACCCNNA TGCACCCNTT GGGTAATNAG GNTTGGGGGT
551 TTGTGCCCGG TAAGC

SEQ ID NO. 30
29.2he.1.t7 Length: 558 nt
1 CGCAAAACGT CANCAGAANG NACTNCCTAA TGCACTAATG AAGGGCGGTA
51 TTAAATCGTA CGTTGAGTTA TTGANCGNAA AATAAAGGAA CCTATTCATG
101 AATGAGCCAA TTTATATTCA TCAATCTAAA GATGATATTG ANGTAGAAAT
151 TGCNATTCAN TATAACTCAG GATATGCCAC AAATCTTTTA ACTTACGCAA
201 ATAACATTCA TACGTATGAN GGTGGTACGC ATGANGACGG ATTCAAACGT
251 GCATTTACGC GTGTCTTAAA TAGTTATGGT TTAAGTAGCA AGATTNTGTA
2301 AGANGGAAAA GNTAGNCTTT CTGGTGAAGN TACACGTGAA GGTATNNCNG
351 CNNTTNTATC TNTCAAACNT GGGGNTCCNC AATTNGGAGG TCAAACGGGG
401 CAAAAATTTG GGNNTTCTGT AGTGCGTCAN GTTGTNGGTN AATTATTCNN
451 NGNGNCTTTT TACNGTTTTN CTTTGNAAAT CCNCNAGTCG GNCGTNCNGT
501 GGTTTNNAAA AGGGTTTTTT GNGGCACGTG NACGTGTTNT TCGGAAAAAA
551 AGCGGGTT

Mutant: NT31
Phenotype: temperature sensitivity
Sequence map: Mutant NT31 is complemented by pMP64, which contains a 1.4 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 36. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the nucleic acid and peptide levels to the *aroE* gene of *B. aphidicola* (Genbank Accession No.

U09230; unpublished as of 1995), which encodes the shikimate-5-dehydrogenase protein (EC 1.1.1.25). Strong similarities also exist at the peptide level to the *aroE* genes from *E. coli* and *P. aeruginosa*. The size and relative position of the predicted AroE ORF within the pMP64 clone is depicted in the restriction map by an arrow.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP64, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP64
SEQ ID NO. 31

pMP64 Length: 1508 nt

```
   1 AGTSGWTCCG TGTGCATAGG TRTGAACTTT GAACCACCAC GTTTAATTTC
  51 ATCGTCACAA ATATCTCCAA AACCAAGCTC GTCGATAATC ATCTGTATCA
 101 TTGTTAATCT GTGCTGAACG TCTATAAAAT CATGGTGCTT TTTCAATGGA
 151 GACATAAAAC TAGGTAAAAA ATAAAATTCA TCTGGCTGTA ATTCATGAAA
 201 TACTTCGCTA GCTACTATCA TATGTGCAGT ATGGATAGGG TTAAACTGAC
 251 CGCCGTAAAG TACTATCTTT TTCATTATTA TGGCAATTCA ATTTCTTTAT
 301 TATCTTTAGA TTCTCTATAA ATCACTATCA TAGATCCAAT CACTTGCACT
 351 AATTCACTAT GAGTAGCTTC GCTTAATGTT TCAGCTAATT CTTTTTTATC
 401 ATCAAAGTTA TTTTGTAGTA CATGTACTTT AATCAATTCT CTGTTTTCTA
 451 ACGTATCATC TATTTGTTTA ATCATATTTT CGTTGATACC GCCTTTTCCA
 501 ATTTGAAAAA TCGGATCAAT ATTGTGTGCT AAACTTCTTA AGTATCTTTT
 551 TTGTTTGCCA GTAAGCATAT GTTATTCTCC TTTTAATTGT TGTAAAACTG
 601 CTGTTTTCAT AGAATTAATA TCAGCATCTT TATTAGTCCA AATTTTAAAG
 651 CTTTCCGCAC CCCTGGTAAA CAAACATATC TAAGCCATTA TAAATATGGT
 701 TTCCCTTGCG CTCTGCTTCC TCTAAAATAG GTGTTTTATA CGGTATATAA
 751 ACAATATCAC TCATTAAAGT ATTGGGAGAA AGATGCTTTA AATTAATAAT
 801 ACTTTCGTTA TTTCCAGCCA TACCCGCTGG TGTTGTATTA ATAACGATAT
 851 CGAATTCAGC TAAATAACTT TTCAGCATCT GCTAATGAAA TTTGGTTTAT
 901 ATTTAAATTC CAAGATTCAA AACGAGCCAT CGTTCTATTC GCAACAGTTA
 951 ATTTGGGCTT TACAAATTTT GCTAATTCAT AAGCAATACC TTTACTTGCA
1001 CCACCTGCGC CCAAAATTAA AATGTATGCA TTTTCTAAAT CTGGATAAAC
1051 GCTGTGCAAT CCTTTAACAT AACCAATACC ATCTGTATTA TACCCTATCC
1101 ACTTGCCATC TTTTATCAAA ACAGTGTTAA CTGCACCTGC ATTAATCGCT
1151 TGTTCATCAA CATAATCTAA ATACGGTATG ATACGTTCTT TATGAGGAAT
1201 TGTGATATTA AAGCCTTCTA ATTCTTTTTT CGAAATAATT TCTTTAATTA
1251 AATGAAAATC TTCAATTGGA ATATTTAAAG CTTCATAAGT ATCATCTAAT
1301 CCTAAAGAAT TAAAATTTGC TCTATGCATA ACGGGCGACA AGGAATGTGA
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1351  AATAGGATTT CCTATAACTG CAAATTTCAT TTTTTTAATC ACCTTATAAA
1401  ATAGAATTTC TTAATACAAC ATCAACATTT TTAGGAACAC GAACGATTAC
1451  TTTAGCCCCT GGTCCTATAG TTATAAAGCC TAGACCAGAG ATCGACCTGC
1501  AGGCAGCA
```

Mutant: NT33a

Phenotype: temperature sensitivity

Sequence map: Mutant NT33a is complemented by pMP67, which contains a 1.8 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 37. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to ORFs of unknown function in *Synechoccocus* sp. (identified as "orf2" in Genbank Accession No. L19521), *M. tuberculosis* (Genbank Accession No. U00024) and *E. coli* (Genbank Accession No. M86305).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP59, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP67

SEQ ID NO. 32

```
pMP67  Length: 1810 nt

  1  CGCGTCTTCC AAATTTCNAA AGCTGTAAAA AGTTATTAAA TCAAATCTTG
 51  CGAATTTGGA TNTAGAGGCA CAATCTGANG TTTATAAAAN TAATGCAGAT
101  AGAGCTTTAA AAGCNTTGTC AAAACGTGAT ATTCAATTTG ATNTCATTTT
151  CTTAGATCCA CCTTATAATA AAGGTCTCAT TGATAAAGCT TTAAAACTAA
201  TTTCAGAGTT TAATTTATTG AAAGAAAATG GTATCATCGT TTGTGAATTT
251  AGCAATCATG AAGAAATAGA TTATCAACCG TTTAATATGA TTAAACGTTA
301  CCATTATGGG TTGACAGACA CATTGTTATT AGAAAAGGGA GAATAGCATG
351  GAACATACAA TAGCGGTCAT TCCGGGTAGT TTTGACCCCA TTACTTATGG
401  TCATTTAGAC ATTATTGAGA GAAGTACAGA TAGATTTGAT GAAATTCATG
451  TCTGTGTTCT TAAAAATAGT AAAAAAGAAG GTACGTTTAG TTTAGAAGAG
501  CGTATGGATT TAATTGAACA ATCTGTTAAA CATTTACCTA ATGTCAAGGT
551  TCATCAATTT AGTGGTTTAC TAGTCGATTA TTGTGAACAA GTAGGAGCTA
601  AAACAATCAT ACGTGGTTTA AGAGCAGTCA GTGATTTTGA ATATGAATTA
651  CGCTTAACTT CMATGAATAA AAAGTTGAAC AATGAAATTG AAACGTTATA
701  TATGATGTCT AGTACTAATT ATTCATTTAT AAGTTCAAGT ATTGTTAAAG
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751   AAGTTGCAGC TTATCGAGCA GATATTTCTG AATTCGTTCC ACCTTATGTT
801   GAAAAGGCAT TGAAGAAGAA ATTTAAGTAA TAAAAATAAC AGTATTTTAG
851   GTTTATCATG GTTTACAATC CTAAAATACT GTTTTCATTT GTTAACGATA
901   TTGCTGTATG ACAGGCGTGT TGAAATCTGT TTGTTGTTGC CCGCTTATTG
951   CATTGTATAT GTGTGTTGCT TTGATTTCAT TTGTGAAGTA ATGTGCATTG
1001  CTTTTGTTAA TATTGGTTAT ATATTGTCTT TCTGGGAACG CTGTTTTTAA
1051  ATGCTTTAAA TATTGTCTGC CACGGTCGTT CATCGCTAAT ACTTTAACTG
1101  CGTGAATGTT ACTCGTAACA TCTGTAGGTT TAATGTTTAA TAATACATTC
1151  ATTAACAGTC TTTGGATATG CGTATATGTA TAACGCTTTG TTTTTAGTAA
1201  TTTTACAAAA TGATGAAAAT CAGTTGCTTC ATAAATGTTA GATTTCAAAC
1251  GATTTTCAAA ACCTTCAGTA ACAGTATAAA TATTTTTTAA TGAATCTGTA
1301  GTCATAGCTA TGATTTGATA TTTCAAATAT GGAAATATTT GATTTAATGT
1351  WATATGAGGT GTTACGTACA AGTGTTGAAT ATCTTTAGGT ACCACATGAT
1401  GCCAATGATC ATCTTGACTA ATGATTGATG TTCTAATAGA TGTACCACTT
1451  SCAAACTGAT GGTGTTGAAT TAATGAATCA TGATGTTGAG CATTTTCTCG
1501  TTTGATAGAA ATTGCATTGA TGTTTTTAGC ATTTTTAGCA ATTGCTTTCA
1551  GGTAACTAAT ACCAAGTATG TTGTTAGGAC TTGCTAGTGC TTCATGATGC
1601  TCTAATAATT CGCTAATGAT ACGAGGGTAG CTTTTACCTT CTTTTACTTT
1651  TNGTGAAAAG GATTCAGATN GTTCAATTTC ATTAATNCTG NGTGCTAATT
1701  GCTTTAANGT TTNGATATCA TTATTTTCAC TACCAAATGC AATGGTATCG
1751  ACACTCATAT AATCNGCGAC TTNAACGGCT AGTTCGGCCA AGGGATCGAC
1801  CGGCAGGCAG
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Mutant: NT33b

Phenotype: temperature sensitivity

Sequence map: Mutant NT33b is complemented by pMP636, which contains a 1.8 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 38. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to the *lepC* gene product, encoding signal peptidase I (EC 3.4.99.36) from *B. caldolyticus* (abbreviated as "Bca" in the sequence map).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP636, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP636

SEQ ID NO. 33

pMP636 Length: 1876 nt

```
   1 TCTGAATGAT CTARACGGAT TAAATTATTT AGCTGGTAAA ACAATCGACG
  51 AAGTTAACAC AAAAGCATTC GAAGGTACAT TATTAGCGCA TACTGATGGT
 101 GGTGTTCCTA ACATGGTAGT GAACATTCCA CAATTAGATG AAGAAACTTT
 151 CGGTTACGTC GTATACTTCT TCGAACTTGC TTGTGCAATG AGTGGATACC
 201 AATTAGGCGT AAATCCATTT AACCAACCTG GTGTAGAAGC ATATAAACAA
 251 AACATGTTCG CATTATTAGG TAAACCTGGT TTTGAAGACT TGAAAAAAGA
 301 ATTAGAAGAA CGTTTATAAA ATACATTACT TCAAAGATTA GTGAAGTTTG
 351 AAAAGATAGA ACTAGACGTT AACTATTTAA AGCATATTTT CGAGGTTGTC
 401 ATTACAAATG TAAAAATGTA ATGACAACCT CGTTTTTATT TATATGCAAG
 451 AACTAGGTTA CTAGCTAATG TGACAAGATG TTWAGAGAAA ATTAAAGATA
 501 AAATAATATC TGCCTTACAA TAATATTGTT ATACTACTAG AGACTGATTT
 551 ATTAGCATGA TTACATGTTA ATGTTTCTTT ACTTAGTAAT TAACTTTRTA
 601 ATGTAARAHT AATTATCTTC ADCCAHAGAA AGGGATTGAT GATTTGTCGT
 651 WTCMTCAATT AGAAGAATGG TTTGAGATAT KTCGACAGTT TGGTTWTTTA
 701 CCTGGATTTA TATTGTTATA TATTAGAGCT NTAATTCCAG TATTTCCTTT
 751 ARCACTCTAT ATTTTAATTA ACATTCAAGC TTATGGACCT ATTTTAGGTA
 801 TATTGATTAG TTGGCTTGGA TTAATTTCTG GAACATTTAC AGTCTATTTG
 851 ATCTGTAAAC GATTGGTGAA CACTGAGAGG ATGCAGCGAA TTAAACAACG
 901 TACTGCTGTT CAACGCTTGA TTAGTTTTAT TGATCGCCAA GGATTAATCC
 951 CATTGTTTAT TTTACTTTGT TTTCCTTTTA CGCCAAATAC ATTAATAAAT
1001 TTTGTAGCGA GTCTATCTCA TATTAGACCT AAATATTATT TCATTGTTTT
1051 GGCATCATCA AAGTTAGTTT CAACAATTAT TTTAGGTTAT TTAGGTAAGG
1101 AAATTACTAC AATTTTAACG CATCCTTTAA GARGGATATT AATGTTAGTT
1151 GGTGTTGGTT GTATTTTGGA TTGTTGGAAA AAAGTTAGAA CAGCATTTTA
1201 TGGGATCGAA AAAGGAGTGA CATCGTGAAA AAAGTTGTAA AATATTTGAT
1251 TTCATTGATA CTTGCTATTA TCATTGTACT GTTCGTACAA ACTTTTGTAA
1301 TAGTTGGTCA TGTCATTCCG AATAATGATA TGYMCCCAAC CCTTAACAAA
1351 GGGGATCGTG TTATTGTWAA TAAAATTAAA GTAACATTTA ATCAATTGAA
1401 TAATGGTGAT ATCATAACAT ATAGGCGTGG TAACGGAGAT ATATACTAGT
1451 CGAATTATTG CCAAACCTGG TCAATCAATG GCGTTTCGTC AGGGACAATT
1501 ATACCGTGAT GACCGACCGG TTGACGCATC TTATGCCAAG AACAGAAAAA
1551 TTAAAGATTT TAGTTTGCGC AATTTTAAAG AATTAGGATG GTGATATTAT
1601 TCCGCCAAAC AATTTTGTTG TGCTAAATGA TCAAGATAAT AACAAGCACG
1651 ATTCAAGACA ATTTGGTTTA ATCGATAAAA AGGATATTAT TGGTAATGTT
1701 AGTTTACGAT ACTATCCTTT TTCAAAATGG ACTGTTCAGT TCAAATCTTA
1751 AAAAGAGGTG TCAAAATTGA AAAAAGAAAT ATTGGAATGG ATTATTTCAA
1801 TTGCAGTCGC TTTTGTCATT TTATTTATAG TAGGTAAATT TATTGTTACG
1851 CCATATACAA TTAAAGGTGA ATCAAT
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Mutant: NT36

Phenotype: temperature sensitivity

Sequence map: Mutant NT36 is complemented by pMP109, which contains a 2.7 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 39. Database searches at both the nucleic acid and peptide levels reveal

identity at one end of the pMP109 clone to the *plaC* gene from *S. aureus* (Genbank Accession No. M63177), encoding a DNA-directed RNA polymerase (EC 2.7.7.6). Since clone pMP109 does not contain the entire *plaC* ORF, the complementation of mutant NT36 by clone pMP109 is not likely to be due to the presence of this gene. Further analysis of clone pMP109 reveals strong similarity at the peptide level to the *dnaG* gene of *L. monocytogenes* (Genbank Accession No. U13165; published in Lupski et al., 1994, *Gene* 151:161-166), encoding DNA primase (EC 2.7.7.-); these similarities also extend to the *dnaG* genes of *L. lactis*, *B. subtilis*, and *E. coli*. The relative size and location of the *dnaG* ORF within clone pMP109 is denoted by an arrow in the sequence map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP109, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP109
SEQ ID NO. 34

pMP109 Length: 2687 nt

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  1  TATGATGATG GTAAAGATCC TAAAGGATTA CCTAAAGCTG ATATTGTTTT
 51  ACTTGGTATT TCGAGAACTT CAAAGACACC ATTATCTCAG TATTTAGCGC
101  ATAAGAGTTA CAAAGTTATG AATGTACCGA TTGTACCAGA AGTGACACCG
151  CCAGATGGCT TATATGATAT TAATCCAAAG AAATGTATCG CACTTAAAAT
201  AAGTGAAGAA AAATTAAATC GCATTAGAAA AGAGCGACTA AAACAATTAG
251  GACTAGGTGA CACAGCTCGA TATGCAACAG AAGCACGAAT TCAAGAAGAA
301  TTGAATTACT TTGAAGAAAT CGTAAGTGAA ATTGGATGTC CTGTCATTGA
351  TGTTTCTCAA AAAGCAATCG AAGAAACAGC AAACGATATA ATCCATTATA
401  TTGAACAAAA TAAATCGAAA TGATTTCATT TTTGTCGAAA ATTAGGTATA
451  ATAGTATAAC TAATGCTTAA TAGGTGATTT AATTTGCGAA TAGATCAATC
501  GATCATTAAT GAAATAAAAG ATAAAACCGA CATTTTAGAC TTGGTAAGTG
551  AATATGTWAA ATTAGAAAAG AGAGGACGCA ATTATATAGG TTTGTGTCCT
601  TTTCATGATG AAAAGACACC TTCATTTACA GTTTCTGAAG ATAAACAAAT
651  TTGTCATTGT TTTGGTTGTA AAAAAGGTGG CAATGTTTTC CAATTTACTC
701  AAGAAATTAA AGACATATTC ATTTGTTGAM GCGGTTAAAG AATTAGGTGG
751  WTAGRGTTAA TGTTTGCTGT AGRTATTGAG GCAMCACAAT CTTWACTCAA
801  ATGTYCAAAT TSCTTCTSRY GRTTTACAAA TGATTGACAW TGCATGGRGT
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851  TAWTACAAGR ATTTTATTAT TACGCTTTAA CAAAGACAGT CGAAGGCGAA
901  CAAGCATTAA CGTACTTACA AGAACGTGGT TTTACAGATG CGCTTATTAA
951  AGAGCGAGGC ATTGGCTTTG CACCCGATAG CTCACATTTT TGTCATGATT
1001 TTCTTCAAAA AAAGGGTTAC GATATTGAAT TAGCATATGA AGCCGGATTA
1051 TWATCACGTA ACGAAGAAAA TTTCAGTTAT TTACGATAGA TTYCGAAAYC
1101 GTATTATGTT YCCTTTGAAA AATGCGCAAG GAAGAATTGT TGGATATTCA
1151 GGTCGAACAT ATACCGGTCA AGAACCAAAA TACTTAAATA GTCCTGAAAC
1201 ACCTATCTTT CAAAAAAGAA AGTTGTTATA CAACTTAGAT AAAGCGCGTA
1251 AATCAATTAG AAAATTAGAT GAAATCGTAT TACTAGAAGG TTTTATGGAT
1301 GTTATAAAAT CTGATACTGC TGGCTTGAAA AACGTTGTTG CAACAATGGG
1351 TACACAGTTG TCAGATGAAC ATATTACTTT TATACGAAAG TTAACATCAA
1401 ATATAACATT AATGTTTGAT GGGGATTTTG CGGGTAGTGA AGCAACACTT
1451 AAAACAGGTY CAAAATTTGT TACAGCAAGG GCTAAATGTR TTTKTTATAC
1501 AATTGCCATC AGGCATGGAT CCGGATGAAT ACATTGGTAA GTATGGCAAC
1551 GATGCATTTM CTGCTTTTST AAAAAATGAC AAAAAGTCAT TTSCACATTA
1601 TAAAGTGAGT ATATTAAAAG ATGAAATTGC ACATAATGAC CTTTCATATG
1651 AACGTTATTT GAAAGAMCTA AGTCATGATA TTTCGCTTAT GAAATCATCG
1701 ATTTTGCAAC AAAAGGCTTT AAATGATGTT GCACCATTTT TCAATGTTAG
1751 TCCTGAGCAA TTAGCTAACG AAATACAATT CAATCAAGCA CCAGCCAATT
1801 ATTATCCAGA AGATGAGTAT GGCGGTTACA TTGAACCTGA GCCAATTGGT
1851 ATGGCACAAT TTGACAATTT GAGCCGTCAA GAAAAAGCGG AGCGAGCATT
1901 TTTAAAACAT TTAATGAGAG ATAAAGATAC ATTTTTAAAT TATTATGAAA
1951 GTGTTGATAA GGATAACTTC ACAAATCAGC ATTTTAAATA TGTATTCGAA
2001 GTCTTACATG ATTTTTATGC GGAAAATGAT CAATATAATA TCAGTGATGC
2051 TGTGCAGTAT GTTAATTCAA ATGAGTTGAG AGAAACACTA ATTAGCTTAG
2101 AACAATATAA TTTGAATGAC GAACCATATG AAAATGAAAT TGATGATTAT
2151 GTCAATGTTA TTAATGAAAA AGGACAAGAA ACAATTGAGT CATTGAATCA
2201 TAAATTAAGG GAAGCTACAA GGATTGGCGA TGTAGAATTA CAAAAATACT
2251 ATTTACAGCA AATTGTTGCT AAGAATAAAG AACGCATGTA GCATGTGATT
2301 TTAAAGAATA ATACGAATAA TGATTATGTC AAAATGTATA AGGGTAAATG
2351 ATAGTTACCG CATTTAAACA ACACTATTGA AAAATAAATA TTGGGATTAG
2401 TTCCAATTTG TAAAATAAAA TTAAAAATAT GGATGAATTA ATTAAGAATT
2451 TAGTTTAAAA TAGCAATATT GAATAAATTT CGAATGTTCA TATTTAAAAT
2501 CGGGAGGCCG TTTCATGTCT GATAACACAG TTAAAATTAA AAAACAAACA
2551 ATTGATCCGA CATTAACATT AGAAGATGTT AAGAAGCAAT TAATTGAAAA
2601 AGGTAAAAAA GAGGGTCATT TAAGTCATGA AGAAATTGCT GAAAAACTTC
2651 AGAATTTTGA TATCGACTCT GATCAAATGG ATGATTT
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Mutant: NT37

Phenotype: temperature sensitivity

Sequence map: Mutant NT37 is complemented by pMP72, which contains a 2.8 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted 40. Database searches at both the nucleic acid and peptide levels reveal a strong similarity at the peptide level to the *glmS* gene of *B. subtilis* (Genbank Accession No. U21932; published in

Morohoshi, F. *et al. J. Bacteriol.* **175** (1993) 6010-6017), which encodes the protein L-glutamine-D-fructose-6-phosphate amidotransferase (EC 2.6.1.16). The relative location and predicted size of this ORF is designated by an arrow in the sequence map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP72, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP72
SEQ ID NO. 35

pMP72 Length: 2800 nt

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   1 NTNAATTAAC ATGCGAGGNC ACCCCTTTAT TGCTACTCCA TACTTCTCAT
  51 AAAATCATAT TAACATAACA CCCTTAATTG TCAGACTATT NAAATAAATA
 101 AAACACTTCA TTTTTACGCA TTTCTGCCAA ATTAAGATGA AGTAAAAGCT
 151 AAGTCGACCT AAAAAAGCAC CCTTCTAGTC GATTAATCTA AAAGGGGTGC
 201 CATATACTTT AATTTTAATA CATGATTGAT TCTAAAAAAG TGAATTATTC
 251 CACAGTAACT GATTTAGCAA GGTTACGTGG TTTATCAACA TCTAAATCTC
 301 TGTGTAATGC TGCATAGTAT GAAATTAATT GTAATGCAAC CACTGATACT
 351 AATGGCGTTA ACAATTCATG TACATGAGGA ATGACATAAG TGTCGCCTTC
 401 TTTTTCAAGA CCCTCCATAG AAATAATACA TGGATGTGCA CCACGTGCTA
 451 CTACCTCTTT AACGTTACCA CGAATTGATA AATTAACTTT CTCTTGTGTT
 501 GCTAAACCTA CAACTGGTGT ACCTTCTTCG ATTAAGGCAA TTGTACCATG
 551 TTTAAGTTCT CCACCAGCAA AACCTTCTGC TTGAATGTAA GAAATTTCTT
 601 TAAGTTTTAA CGCACCTTCT AAACTTACGT TATAGTCAAT AGTACGTCCG
 651 ATAAANAATG CATTGCGTGT TGTTTCTAAG AAATCTGTAG CAATTTGTTC
 701 CATAATTGGT GCATCGTCAA CAATTGCTTC TATTGCTGTT GTTACTTTTG
 751 CTAATTCTCT CAATAAATCA ATATCTGCTT CACGACCATG CTCTTTTGCA
 801 ACGATTTGAG ACAAGAWTGA TAATACTGCA ATTTGTGCAG WATAWGCTTT
 851 TGTAGATGCA ACTGCGAWTT CAGGGACCCG CGTGTAATAA CAATGTGTGG
 901 TCTGCTTCAC GTTGATAAAG TTGAACCTGC AACATTAGTG ATTGTTAATG
 951 AWTTATGAMC TAATTTATTA GTTWCAACTA AATACGGCGC GGCTATCTGG
1001 CAGTTTCACC TGATTGAGAA ATATAAACGA ACAATGGTTT TTAAGATAAT
1051 AATGGCATGT TGTAGACAAA CTCTGATGCA ACGTGTACTT CAGTTGGTAC
1101 GCCAGCCCAT TTTTCTAAAA ATTCTTTACC TACTAAACCT GCATGGTAGC
1151 TTGTACCTGC TGCAATAACG TAAATGCGGT CTGCTTCTTT AACATCATTG
1201 ATGATGTCTT GATCAATTTT CAAGTTACCT TCTGCATCTT GATATTCTTG
1251 AATAATACGA CGCATTACTG CTGGTTGTTC ATGAATTTCT TTTAACATGT
1301 AGTGTGCATA AACACCTTTT TCAGCATCTG ATGCATCAAT TTCAGCAATA
1351 TATGAATCAC GTTCTACAAC GTTTCCATCT GCATCTTTAA TAATAACTTC
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1401 ATCTTTTTTA ACAATAACGA TTTCATGGTC ATGGRTTTCT TTATATTCGC
1451 TTGTCACTTG TAACATTGCA AGTGCGTCTG ATGCGATAAC ATTGAAACCT
1501 TCACCAACAC CTAATAATAA TGGTGATTTA TTTTTAGCAA CATAGATTGT
1551 GCCTTTGHCT TCAGCATCTA ATAAACCTAA TGCATATGAA CCATGTAATA
1601 ATGACACAAC TTTTGTAAAT GCTTCTTCAG TTGAAAGTCC TTGATTTGAA
1651 AAGTATTCAA CTAATTGAAC GATAACTTCT GTATCTGTTT CTGAAATGAA
1701 TGATACACCT TGTAAGTATT CACCTTTTAA CTCTTCATAG TTTTCAATAA
1751 CACCGTTATG AACTAGAGTA AAACGGCCAT TTGATGATTG ATGTGGATGA
1801 GAGTTTTCAT GATTCGGTAC ACCGTGTGTT GCCCAACGTG TGTGACCGAT
1851 TCCAACAGGT CCATTCAAAA TCGCTACTAT CAGCAACTTT ACGTAATTCT
1901 GCAATACGAC CTTTTTCTTT AAATACAGTT GTATTATCAT YATTTACTAC
1951 TGCGATACCT GCAGAGTCAT AACCTCTGTA TTCTAATTTT TCTACAACCT
2001 TTTAATAATA ATTTCTTTGG CATTATCATA GCCAATATAA CCAACAATTC
2051 CACACATAAC GACATTTTCC TCCATATTGG AATAGTACGS GTAAATTATG
2101 ATTTATTGCC GATAATTTAG ATTGACAATC TGCTTTCATA ATATAAATAG
2151 GAACATGCTA TCATCGCATT CATCCATAAC AAATTAAGCA TAGTTATTTT
2201 TACAACTATA CAAATTGCTC ACACTGTACT TTCCATATTA ATATTTTTTA
2251 TATTCAATTT CTGGCGATCT TATTAACTTT GTCCATTAAG TCACCCTAAT
2301 GTTTTACTTA ATAAGCTAAC GAATGAGCCA CATCCGGGAT AGCATCCGCC
2351 GATCTATTCG ATCACTATCC TCTTCGTCTA CAAATACATA TATTGCACTC
2401 TATAAAGGCC ACTCATATAT TAACCTTTAA TCTTCAAATA CAAATATTTA
2451 TTTGCACAGG CGCTTTAACT GTACTGCCGA ACTTTCCCCC TTTCCATTAA
2501 TCATTATTGT ACAACGGTGT TGTTTTGTTT TGCAAATATT TTCACAATAA
2551 AATTTTAAAA ATCCTAAAAC AATTTTTTTG TTTTACTTTT TCAAAATATC
2601 TATACTGTCA CATTGATGAC ACTTTATTTA ATTTTGTCAC ATTTATTTTG
2651 ACAAAGTTGA TTTTTGTTTA TATTGAGTAA CAAGTAACCT CTCTATACAC
2701 TATATATAGT CACATATATT AAAAAAGAGG TGTAAACATG TCACAAACTG
2751 AAGAGAAAAA AGGAATTGGT CGTCGTGTTC AAGCATTTGG ATCGACCGCA
```

Mutant: NT41/64

Phenotype: temperature sensitivity

Sequence map: Mutants NT41 and NT64 are complemented by pMP98, which contains a 2.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 41. Database searches at both the nucleic acid and peptide levels reveal identity at both the peptide and nucleic acid levels to the C-terminal fragment of the *pcrA* gene from *S. aureus* (Genbank Accession No. M63176; published in Iordanescu, S.M. *et al. J. Bacteriol.* 171 (1989) 4501-4503), encoding DNA helicase (EC 3.6.1.-). Since only a small portion of the C-terminal fragment of the helicase protein is contained within clone pMP98, the *pcrA* gene is unlikely to be responsible for restoring a wild-type phenotype to mutants NT41 and 64. Further analysis reveals

strong peptide level similarity to the *lig* gene of *E. coil*(Genbank Accession No. M30255; published in Ishino, Y. *et al.*, *Mol. Gen. Genet.* **204** (1986) 1-7), encoding the protein DNA ligase (EC 6.5.1.2). The relative location and predicted size of the ORF encoding the putative *S. aureus lig* gene is depicted by an arrow in the sequence map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP98, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP98
SEQ ID NO. 36

```
pMP98  Length: 2934 nt

   1  CATGAAATGC AAGAAGAACG TCGTATTTGT TATGTAGCAA TTACAAGGGC
  51  TGAAGAGGTG TTATATATCA CTCATGCGAC ATCAAGAATG TTATTTGGTC
 101  GCCCTCAGTC AAATATGCCA TCCAGATTTT TAAAGGAAAT TCCAGAATCA
 151  CTATTAGAAA ATCATTCAAG TGGCAAACGA CAAACGATAC AACCTAAGGC
 201  AAAACCTTTT GCTAAACGCG GATTTAGTCA ACGAACAACG TCAACGAAAA
 251  AACAAGTATT GTCATCTGAT TGGAATGTAG GTGACAAAGT GATGCATAAA
 301  GCCTGGGGAG AAGGCATGGT GAGTAATGTA AACGAGAAAA ATGGCTCAAT
 351  CGAACTAGAT ATTATCTTTA AATCACAAGG GCCAAAACGT TTGTTAGCGC
 401  AATTTGCACC AATTGAAAAA AAGGAGGATT AAGGGATGGC TGATTTATCG
 451  TCTCGTGTGA ACGRDTTACA TGATTTATTA AATCAATACA GTTATGAATA
 501  CTATGTAGAG GATAATCCAT CTGTACCAGA TAGTGAATAT GACAAATTAC
 551  TTCATGAACT GATTAAAATA GAAGAGGAGC ATCCTGAGTA TAAGACTGTA
 601  GATTCTCCAA CAGTTAGAGT TGGCGGTGAA GCCCAAGCCT CTTTCAATAA
 651  AGTCAACCAT GACACGCCAA TGTTAAGTTT AGGGAATGCA TTTAATGAGG
 701  ATGATTTGAG AAAATTCGAC CAACGCATAC GTGAACAAAT TGGCAACGTT
 751  GAATATATGT GCGAATTAAA AATTGATGGC TTAGCAGTAT CATTGAAATA
 801  TGTTGATGGA TACTTCGTTC AAGGTTTAAC ACGTGGTGAT GGAACAACAG
 851  GTTGAAGATA TTACCGRAAA TTTAAAAACA ATTCATGCGA TACCTTTGAA
 901  AATGAAAGAA CCATTAAATG TAGAAKTYCG TGGTGAAGCA TATATGCCGA
 951  GACGTTCATT TTTACGATTA AATGAAGAAA AAGAAAAAAA TGATGAGCAG
1001  TTATTTGCAA ATCCAAGAAA CGCTGCTGCG GGATCATTAA GACAGTTAGA
1051  TTCTAAATTA ACGGCAAAAC GAAAGCTAAG CGTATTTATA TATAGTGTCA
1101  ATGATTTCAC TGATTTCAAT GCGCGTTCGC AAAGTGAAGC ATTAGATGAG
1151  TTAGATAAAT TAGGTTTTAC AACGAATAAA AATAGAGCGC GTGTAAATAA
1201  TATCGATGGT GTTTTAGAGT ATATTGAAAA ATGGACAAGC CAAAGAAGAG
1251  TTCATTACCT TATGATATTG ATGGGATTGT TATTAAGGTT AATGATTTAG
1301  ATCAACAGGA TGAGATGGGA TTCACACAAA AATCTCCTAG ATGGGCCATT
1351  GCTTATAAAT TTCCAGCTGA GGAAGTAGTA ACTAAATTAT TAGATATTGA
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1401  ATTAAGTATT GGACGAACAG GTGTAGTCAC ACCTACTGCT ATTTTAGAAC
1451  CAGTAAAAGT AGCTGGTACA ACTGTATCAA GAGCATCTTT GCACAATGAG
1501  GATTTAATTC ATGACAGAGA TATTCGAATT GGTGATAGTG TTGTAGTGAA
1551  AAAAGCAGGT GACATCATAC CTGAAGTTGT ACGTAGTATT CCAGAACGTA
1601  GACCTGAGGA TGCTGTCACA TATCATATGC CAACCCATTG TCCAAGTTGT
1651  GGACATGAAT TAGTACGTAT TGAAGGCGAA GTTAGCACTT CGTTGCATTA
1701  ATCCAAAATG CCAAGCACAA CTTGTTGAAG GATTGATTCA CTTTGTATCA
1751  AGACAAGCCA TGAATATTGA TGGTTTAGGC ACTAAAATTA TTCAACAGCT
1801  TTATCAAAGC GAATTAATTA AAGATGTTGC TGATATTTTC TATTTAACAG
1851  AAGAAGATTT ATTACCTTTA GACAGAATGG GGCAGAAAAA AGTTGATAAT
1901  TTATTAGCTG CCATTCAACA AGCTAAGGAC AACTCTTTAG AAAATTTATT
1951  ATTTGGTCTA GGTATTAGGC ATTTAGGTGT TAAAGCGAGC CAAGTGTKAG
2001  CAGAAAAATA TGAAACGATA GATCGATTAC TAACGGTAAC TGAAGCGGAA
2051  TTAGTAGAAT TCATGATATA GGTGATAAAG TAGCGCAATC TGTAGTTACT
2101  TATTTAGCAA ATGAAGATAT TCGTGCTTTA ATTCCATAGG ATTAAAAGAT
2151  AAACATGTTA ATATGATTTA TGAAGGTATC CAAAACATCA GATATTGAAG
2201  GACATCCTGA ATTTAGTGGT AAAACGATAG TACTGACTGG TAAGCTACAT
2251  CCAAATGACA CGCAATGAAG CATCTAAATG GCTTGCATCA CCAAGGTGCT
2301  AAAGTTACAA GTAGCGTTAC TAAAAATACA GATGTCGTTA TTGCTGGTGA
2351  AGATGCAGGT TCAAAATTAA CAAAAGCACA AAGTTTAGGT ATTGAAATTT
2401  GGACAGAGCA ACAATTTGTA GATAAGCAAA ATGAATTAAA TAGTTAGAGG
2451  GGTATGTCGA TGAAGCGTAC ATTAGTATTA TTGATTACAG CTATCTTTAT
2501  ACTCGCTGCT TGTGGTAACC ATAAGGATGA CCAGGCTGGA AAAGATAATC
2551  AAAAACATAA CAATAGTTCA AATCAAGTAA AAGAAATTGC AACGGATAAA
2601  AATGTACAAG GTGATAACTA TCGTACATTG TTACCATTTA AAGAAAGCCA
2651  GGCAAGAGGA CTTTTACAAG ATAACATGGC AAATAGTTAT AATGGCGGCG
2701  ACTTTGAAGA TGGTTTATTG AACTTAAGTA AAGAAGTATT TCCAACAGAT
2751  AAATATTTGT ATCAAGATGG TCAATTTTTG GACAAGAAAA CAATTAATGC
2801  CTATTTAAAT CCTAAGTATA CAAAACGTGA AATCGATAAA ATGTCTGAAA
2851  AAGATAAAAA AGACAAGAAA GCGAATGAAA ATTTAGGACT TAATCCATCA
2901  CACGAAGGTG AAACAGATCG ACCTGCAGKC ATGC
```

Mutant: NT42

Phenotype: temperature sensitivity

Sequence map: Mutant NT42 is complemented by pMP76, which contains a 2.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 42.' Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to ORFs of unknown function in *B. subtilis* (Genbank Accession No. Z38002; characterization of the Ipc29D polypeptide is unpublished as of 1995). Strong similarity is also noted to the SUA5 protein from the yeast *S. cerevisiae*, which is described as being essential for normal growth (published in Na, J.G. *et al. Genetics* **131** (1992) 791-801).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP76, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP76
SEQ ID NO. 37

```
pMP76  Length: 2515 nt

   1  CSYCGGWACC CGGGGATCCT CTAGAGTCGA TCGTTCCAGA ACGTATTCGA
  51  ACTTATAATT ATCCACAAAG CCGTGTAACA GACCATCGTA TAGGTCTAAC
 101  GCTTCAAAAA TTAGGGCAAA TTATGGAAGG CCATTTAGAA GAAATTATAG
 151  ATGCACTGAC TTTATCAGAG CAGACAGATA AATTGAAAGA ACTTAATAAT
 201  GGTGAATTAT AAAGAAAAGT TAGATGAAGC AATTCATTTA ACACAACAAA
 251  AAGGGTTTGA ACAAACACGA GCTGAATGGT TAATGTTAGA TGTATTTCAA
 301  TGGACGCGTA CGGACTTTGT AGTCCACATG CATGATGATA TGCCGAAAGC
 351  GATGATTATG AAGTTCGACT TAGCATTACA ACGTATGTTA TTAGGGAGAG
 401  CCTATACAGT ATATAGTTGG CTTTGCCTCA TTTTATGGTA GAACGTTTGA
 451  TGTAAACTCA AATTGTTTGA TACCAAGACC TGAAACTGAA GAAGTAATGT
 501  TGCATTTCTT ACAACAGTTA GAAGATGATG CAACAATCGT AGATATCGGA
 551  ACGGGTAGTG GTGTACTTGC AATTACTTTG AAATGTTGAA AAGCCGGATT
 601  TAAATGTTAT TGCTACTGAT ATTTCACTTG AAGCAATGAA TATGGCTCCG
 651  TAATAATGCT GAGAAGCATC AATCACAAAT ACAATTTTTA ACAGGGGATG
 701  CATTAAAGCC CTTAATTAAT GAAGGTATCA AKTTGAACGG CTTTGATATC
 751  TAATCCMCCA TATATAGATG AAAAAGATAT GGTTACGATG TCTCCMACGG
 801  TTACGARATT CGAACCACAT CAGGCATTGT TTGCAGATAA CCATGGATAT
 851  GCTATTTATG AATCAATCAT GGAAGATTTA CCTCACGTTA TGGAAAAAGG
 901  CAGCCCAGTT GTTTTTGAAA TTGGTTACAA TCAAGGTGAG GCACTTAAAT
 951  CAATAATTTT AAATAAATTT CCTGACAAAA AAATCGACAT TATTAAAGAT
1001  ATAAATGGCC ACGATCGAAT CGTCTCATTT AAATGGTAAT TAGAAGTTAT
1051  GCCTTTGCTA TGATTAGTTA AGTGCATAGC TTTTTGCTTT ATATTATGAT
1101  AAATAAGAAA GGCGTGATTA AGTTGGATAC TAAAATTTGG GATGTTAGAG
1151  AATATAATGA AGATTTACAG CAATATCCTA AAATTAATGA AATAAAAGAC
1201  ATTGTTTTAA ACGGTGGTTT AATAGGTTTA CCAACTGAAA CAGTTTATGG
1251  ACTTGCAGCA AATGCGACAG ATGAAGAAGC TGTAGCTAAA ATATATGAAG
1301  CTAAAGGCCG TCCATCTGAC AATCCGCTTA TTGTTCATAT ACACAGTAAA
1351  GGTCAATTAA AAGATTTTAC ATATACTTTG GATCCACGCG TAGAAAAGTT
1401  AATGCAGGCA TTCTGGCCGG GCCCTATTTC GTTTATATTG CCGTTAAAGC
1451  TAGGCTATCT ATGTCGAAAA GTTTCTGGAG GTTTATCATC AGTTGCTGTT
1501  AGAATGCCAA GCCATTCTGT AGGTAGACAA TTATTACAAA TCATAAATGA
1551  ACCTCTAGCT GCTCCAAGTG CTAATTTAAG TGGTAGACCT TCACCAACAA
1601  CTTTCAATCA TGTATATCAA GATTTGAATG GCCGTATCGA TGGTATTGTT
1651  CAAGCTGAAC AAAGTGAAGA AGGATTAGAA AGTACGGTTT TAGATTGCAC
1701  ATCTTTTCCT TATAAAATTG CAAGACCTGG TTCTATAACA GCAGCAATGA
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1751 TTACAGAAAT AMTTCCGAAT AGTATCGCCC ATGCTGATTA TAATGATACT
1801 GAACAGCCAA TTGCACCAGG TATGAAGTAT AAGCATTACT CAACCCAATA
1851 CACCACTTAC AATTATTACA GATATTGAGA GCAAAATTGG AAATGACGGT
1901 AAAGATTRKW MTTCTATAGC TTTTATTGTG CCGAGTAATA AGGTGGCGTT
1951 TATACCAAGT GARSCGCAAT TCATTCAATT ATGTCAGGAT GMCAATGATG
2001 TTAAACAAGC AAGTCATAAT CTTTATGATG TGTTACATTC ACTTGATGAA
2051 AATGAAAATA TTTCAGCGGC GTATATATAC GGCTTTGAGC TGAATGATAA
2101 TACAGAAGCA ATTATGAATC GCATGTTAAA AGCTGCAGGT AATCACATTA
2151 TTAAAGGATG TGAACTATGA AGATTTTATT CGTTTGTACA GGTAACACAT
2201 GTCGTAGCCC ATTAGCGGGA AGTATTGCAA AAGAGGTTAT GCCAAATCAT
2251 CAATTTGAAT CAAGAGGTAT ATTCGCTGTG AACAATCAAG GTGTTTCGAA
2301 TTATGTTGAA GACTTAGTTG AAGAACATCA TTTAGCTGAA ACGACCTTAT
2351 CGCAACAATT TACTGAAGCA GATTTGAAAG CAGATATTAT TTTGACGATG
2401 TCGTATTCGC ACAAAGAATT AATAGAGGCA CACTTTGGTT TGCAAAATCA
2451 TGTTTTCACA TTGCATGAAT ATGTAAAAGA AGCAGGAGAA GTTATAGATC
2501 GACCTGCAGG CATGC

Mutant: NT47

Phenotype: temperature sensitivity

Sequence map: Mutant NT47 is complemented by pMP639, which contains a 2.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 43, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to two hypothetical ORFs of unknown function, one from *K. pneumonia* and one from *Synechocystis spp*. (abbreviated as "Kpn" and "Scy" in the diagram below. Experiments are currently underway to determine which ORF (or both) is an essential gene. The relative orientation and predicted size of these uncharacterized ORFs with respect to the partial restriction map of clone pMP639 are depicted by arrows in the map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP639, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP639

SEQ ID NO. 38

pMP639 Length: 2635 nt

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   1 ATTCTCTGTG TTGGGGCCCC TGACTAGAGT TGAAAAAAGC TTGTTGCAAG
  51 CGCATTTTCA TTCAGTCAAC TACTAGCAAT ATAATATTAT AGACCCTAGG
 101 ACATTGATTT ATGTCCCAAG CTCCTTTTAA ATGATGTATA TTTTTAGAAA
 151 TTTAATCTAG ACATAGTTGG AAATAAATAT AAAACATCGT TGCTTAATTT
 201 TGTCATAGAA CATTTAAATT AACATCATGA AATTCGTTTT GGCGGTGAAA
 251 AAATAATGGA TAATAATGAA AAAGAAAAAA GTAAAAGTGA ACTATTAGTT
 301 GTAACAGGTT TATCTGGCGC AGGTAAATCT TTGGTTATTC AATGTTTAGA
 351 AGACATGGGA TATTTTTGTG TAGATAATCT ACCACCAGTG TTATTGCCTA
 401 AATTTGTAGA GTTGATGGAA CAAGGGAAAT CCATCCTTAA GAAAAAGTGG
 451 CAATTGCAAT TGATTTAAGA RGTAAGGAAC TATTTAATTC ATTAGTTGCA
 501 GTAGTGGATA AAGTTCAAAA GTTGAAAGTG ACGTCATCAT TGATGTTATG
 551 TTTTTAGAAG CAAGTACTGA AAAATTAATT TCAAGATATA AGGAAACGCG
 601 TCCKTGCACA TCCTTTGATG GAACAAGGTT AAAAGATCGT TAATCAATGC
 651 MATTAATGAT GAGCGAGAGC ATTTGTCTCA AATTAGAAGT ATAGCTAATT
 701 TTGTTATAGA TAACTACAAA GTTATCACCT AAAGAATTAA AAGAACGCAT
 751 TCGTCGATAC TATGAAGATG AAGAGTTTGA AACTTTTACA ATTAATGTCA
 801 CAAGTTTCGG TTTTAAACAT GGGATTCAGA TGGATGCAGA TTTAGTATTT
 851 GATGTACGAT TTTTACCAAA TCCATATTAT GTAGTAGATT TAAGACCTTT
 901 AACAGGATTA GATAAAGACG TTTATAATTA TGTTATGAAA TGGAAAGAGA
 951 CGGAGATTTT TCTTTGAAAA ATTAACTGAT TTGTTAGATT TTATGATACC
1001 CGGGTWTAAA AAAGAAGGGA AATCTCAATT AGTAATTGCC ATCGGTTGTA
1051 CGGGTGGGAC AACATCGATC TGTAGCATTA GCAGAACGAC TAGGTWATTA
1101 TCTAAATGAA GTWTTTGAAT ATAATGTTTA TGTGCATCAT AGGGACGCAC
1151 ATATTGAAAG TGGCGAGAAA AAATGAGACA AATAAAAGTT GTACTTATCG
1201 GGTGGTGGCA CTGGCTTATC AGTTATGGCT AGGGGATTAA GAGAATTCCC
1251 AATTGATATT ACGGCGATTG TAACAGTTGC TGATAATGGT GGGAGTACAG
1301 GGAAAATCAG AGATGAAATG GATATACCAG CACCAGGAGA CATCAGAAAT
1351 GTGATTGCAG CTTTAAGTGA TTCTGAGTCA GTTTTAAGCC AACTTTTTCA
1401 GTATCGCTTT GAAGAAAATC AAATTAGCGG TCACTCATTA GGTAATTTAT
1451 TAATCGCAGG TATGACTAAT ATTACGAATG ATTTCGGACA TGCCATTAAA
1501 GCATTAAGTA AAATTTTAAA TATTAAAGGT AGAGTCATTC CATCTACAAA
1551 TACAAGTGTG CAATTAAATG CTGTTATGGA AGATGGAGAA ATTGTTTTTG
1601 GAGAAACAAA TATTCCTAAA AAACATAAAA AAATTGATCG TGTGTTTTTA
1651 GAACCTAACG ATGTGCAACC AATGGAAGAA GCAATCGATG CTTTAAGGGA
1701 AGCAGATTTA ATCGTTCTTG GACCAGGGTC ATTATATACG AGCGTTATTT
1751 CTAACTTATG TTKTGAATGG TATTTCAGAT GCGTTWATTC ATTCTGATGC
1801 GCCTAAGCTA TATGTTTCTA ATGTGATGAC GCAACCTGGG GAAACAGATG
1851 GTTATAGCGT GAAAGATCAT ATCGATGCGA TTCATAGACA AGCTGGACAA
1901 CCGTTTATTG ATTATGTCAT TTGTAGTACA CAAACTTTCA ATGCTCAAGT
1951 TTTGAAAAAA TATGAAGAAA AACATTCTAA ACCAGTTGAA GTTAATAAGG
2001 CTGAACTKGA AAAAGAAAGC ATAAATGTAA AAACATCTTC AAATTTAGTT
2051 GAAATTTCTG AAAATCATTT AGTAAGACAT AATACTAAAG TGTTATCGAC
2101 AATGATTTAT GACATAGCTT TAGAATTAAT TAGTACTATT CCTTTCGTAC
2151 CAAGTGATAA ACGTAAATAA TATAGAACGT AATCATATTA TGATATGATA
2201 ATAGAGCTGT GAAAAAAATG AAAATAGACA GTGGTTCTAA GGTGAATCAT
2251 GTTTTAAATA AGAAAGGAAT GACTGTACGA TGAGCTTTGC ATCAGAAATG
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2301  AAAAATGAAT TAACTAGAAT AGACGTCGAT GAAATGAATG CAAAAGCAGA
2351  GCTCAGTGCA CTGATTCGAA TGAATGGTGC ACTTAGTCTT TCAAATCAAC
2401  AATTTGTTAT AAATGTTCAA ACGGAAAATG CAACAACGGC AAGACGTATT
2451  TATTCGTTGA TTAAACGTGT CTTTAATGTG GAAGTTGAAA TATTAGTCCG
2501  TAAAAAAATG AAACTTAAAA AAAATAATAT TTATATTTGT CGTACAAAGA
2551  TGAAAGCGAA AGAAATTCTT GATGAATTAG GAATTTTAAA AGACGGCATT
2601  TTTACGCATG AAATTGATCG ACCTGCAGGC ATGCA
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Mutant: NT51

Phenotype: temperature sensitivity

Sequence map: Mutant NT51 is complemented by pMP86, which contains a 1.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 44 (there are no apparent restriction sites for EcoR I, Hind III, or BamH I). Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to an ORF of undetermined function in *H. influenzae* (Genbank Accession No. U32702):

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP86, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP86
SEQ ID NO. 39

pMP86 Length: 1952 nt

```
  1  TGCATGTACA GCAGGCTCTA CACAACCGTC GCATGTTTTA GATGCAATGT
 51  TCGAAGATGA GGAGCGATCA AATCATTCGA TTCGATTTAG TTTTAACGAA
101  TTGACTACTG AAAATGAAAT TAATGCAATT GTAGCTGAAA TTCATAAAAT
151  ATATTTTAAA TTTAAGGAGG AGTCATAATT GTCAAATAAA GATATAACGT
201  GTTGTCGTTG GTATGTCAGG CGGTGTAGAT AGTTCTGTAA CAGCCCACGT
251  CTTAAAAGAA CAAGGTTATG ATGTCATTGG CATATTTATG AAAAACTGGG
301  ATGACACTGA CGAAAATGGC GTATGTACTG CAACTGAAGA TTACAACGAT
351  GTTATTGAAG TGTGTAATCA AATTGGCATT CCGTATTACG CTGTTAATTT
401  TGAAAAAGAA TATTGGGATA AAGTCTTTAC GTATTTCTTA GATGAATACA
451  AAAAAGGTCG TACTCCAAAT CCAGACGTTA TGTGTAATAA AGAAATTAAG
501  TTTAAAGCCT TTTTAGATCA TGCGATGAAT TTAGGTGCAG ATTATGTAGC
551  AACAGGACAT TACGCACGCA TACATCGTCA TGAASRTGGT CATGTTGAAA
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601  TGTTACGTGG TGTAGATAAT AATAAAGATC ARACATACTK CWKGMATGCA
651  AKTATCTCAA CAACAACTTT CAAAAGTGAT GTTCCCAATT GGCGACATCG
701  AAAAGAGTGA AGTGCGTCGA ATTGCTGAAG AACAAGGACT TGTTACTGCT
751  AAGAAAAAAG ATTCTACAGG CATTTGTTTT ATCGGCGAAA AAAACTTTAA
801  AACATTTTTA TCACAATATT TACCTGCACA ACCGGGTGAT ATGATAACAC
851  TTGATGGTAA GAAAATGGGT AAACATAGTG GTTTGATGTA TTACACAATA
901  GGACAAAGAC ATGGATTAGG TATAGGTGGG AGATGGCGAT CCTTGGTTTG
951  TTGTCGGTAA AAACCTAAAA GATAATGTTT TATATGTWGA ACAAGGATCC
1001 ATCACGATGC ATTATACAGT GATTACTTAA TTGCTTCAGA CTATTCATTT
1051 GTAAATCCCA GAAGATAATG ACTTAGATCA AGGTTTTGAA TGTACAGCTA
1101 AATTTAGATA TCGCCAAAAA GATACGAAAG TTTTTGTGAA ACGTGAAAAA
1151 CGACCATGCA CTACGTGTTA CTTTTGCTGA GCCAGTAAGA GCAATCACAC
1201 CTGGACAAGC AGTTGTTTTT TATCAAGGTG ATGTGTTGTC TTGGTGGTGC
1251 AACAATTGAC GATGTKTTCA AAAATGAAGG TCAATTAAAT TATGTTGTAT
1301 ANACAATGGC AACAATAAAT TACTTATTTG AAGTTTCNAC GTTGAAAATG
1351 ACGAAAGACA GTTTTTGATG AGAATAATTC ATGAGGATAG AGTCTGGGAC
1401 ATCACAATGT CCTAGGCTCT ACAATGTTAT ATKGGCGGGA CCACAACATA
1451 GAGAATTTCG TAAAGAAATT CWACAGGCAA TGCCAGTTGG GGATAACGAA
1501 TTTAATTTTG TTAAAATATC ATTTCTGTCC CACTCCCTAT GCATGAATCT
1551 AATTATGTAT TCTTATTTTT AAGTACATAA TAGTGGTGGC TAATGTGGAA
1601 GAACCATTAC ATAATAAACC GTTAATGGTT CTTAAGCATT TYTATTCCAT
1651 TCCCGCTTTT TCATGAATGA AGATGATATT AGATTATATT TTATTCGTTG
1701 TTAAGTGATT CGAGACATAC AATTTATCAA GATGTTTATA ATTGATGAGA
1751 AATGAGGTTC GTAAATGATA GATCAACAAA CAATTTATCA ATACATACAA
1801 AATGGAAAAA TAGAAGAAGC GTTACAAGCA TTGTTCGGAA ATATCGAAGA
1851 AAATCCTACA ATTATTGAAA ATTATATTAA TGCTGGTATC GTACTTGCTG
1901 ATGCGAATGA GATTGAAAAG GCAGAGCGTT TTTTCCAAAA AGCTTTAACA
1951 AT
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Mutant: NT52

Phenotype: temperature sensitivity

Sequence map: Mutant NT52 is complemented by pMP87, which contains a 2.3 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 45. Database searches at both the nucleic acid and peptide levels strong peptide-level similarity to the *kimE* gene product, encoding mevalonate kinase (EC 2.7.1.36), from *M. thermoautotrophicum* (abbreviated as "Mth" in the sequence map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP87, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence

contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP87

SEQ ID NO. 40

pMP87 Length: 2273 nt

1 TAACCAATAT TGATAAAACC TTGATGTGTT TCGTGTCAAT GACATACCAT
51 ATCGACTAGG TACCTTTTTA GAATGTTGAT TAATCACAAC AAATATCATG
101 GCAAGGTCAT CTTCAAAATG ATTCGATTCA AGTGGAACGG CATATGACGT
151 CTCATCACTA TACCCTTTTT CCCATTCTGC AAATCCACCA TAAATACTAC
201 GCGACGCAGA ACCCGAACCA ATTCGCGCCA ATCTCGATAA ATCCTTATCT
251 GACAGCTGCA TGTCTAGCGC TTGATTACAA GCTGCTGCTA AAGCTGCATA
301 TGCGCTTGCC GATGAAGCCA ACCCTGCTGC TGTTGGTACA AAATTGTCGC
351 TTTCAATTTC TGCATACCAA TCGATGCCAG CTCTATTTCT GACAATATCC
401 ATATATTTTG AAATTTTCTC TAATTCTTTG CCACTAACCT TTTCACCATT
451 CAACCAAAAT TGATCCTGTG TTAACTGGTC GTTAAAAGTG ACTTTCGTTT
501 CAGTGTWAAA TTTTTCTAAT GTWACAGATA TGCTATTATT CATTGGAATG
551 ATTAGTGCTT CATCTTTTTT ACCCCAATAT TTTATAAGTG CAATATTCGT
601 ATGTGCACGT GCTTTGCCAC TTTTAATCAA CGCATTAACC TCCTAAATTC
651 TCAATCCAAG TATGTGCTGC ACCAGCTTTT TCTACAGCTT TTACAATATT
701 TTTCGCTGTT GGTAAATCTT TGGCAAGCAA TAACATACTT CCACCACGAC
751 CAGCGCCAGT AAGTTTTCCA GCAATCGCAC CATTTTCTTT ACCAATTTTC
801 ATTAATTGTT CTATTTTATC ATGACTAACT GTCAACGCCT TTAAATCCGC
851 ATGACATTCA TTAAAAATAT CCGCTAAGGS TTCAAAGTTA TGATGTTCAA
901 TCACATCACT CGCACGTAAA ACTAACTTAC CGATATGTTT TACATGTGAC
951 ATGTACTGAG GGTCCTCACA AAGTTTATGA ACATCTTCTA CTGCTTGTCT
1001 TGTTGAACCT TTCACACCAG TATCTATAAC AACCATATAG CCGTCTAAAC
1051 TTAACGTTTT CAACGTTTCA GCATGACCTT TTTGGAACCA AACTGGTTTG
1101 CCTGATACAA TCGTTTGCGT ATCAATACCA CTTGGTTTAC CATGTGCAAT
1151 TTGCTCTGCC CAATTAGCCT TTTCAATGAG TTCTTCTTTC GTTAATGATT
1201 TCCCTAAAAA ATCATAACTT GCACGAACAA AAGCAACCGC GACAGCTGCA
1251 CTCGATCCTA ATCCACGTGA TGGTGGTAAA TTCGTTTGGA TCGTTACTGC
1301 TAGCGGCTCT GTAATATTAT TTAATTCTAC AAAACGGTTC ACCAAAGAMT
1351 TAAGATGGTC AGGCGCATCA TATAAACATA CCATCGTAAA ACATCGCTTT
1401 TAATAGAGGA ATAGTTCCCG CTCTCTAAGG TTCTATTAAA ACTTTGATTT
1451 TAACCGGCGT TAAACGGTAC TGCAATAGCA GGCTCTCCAA ATGTAACAGC
1501 ATGTTCTCCT ATTAAAATAA TCTTACCTGT CGATTCCCCA TATCCTTTTC
1551 TTGTCATGTC AATATCACCT TTTATATTTA TCCTAWACTT GATTCATTAT
1601 TTTTATTTAT TAGTAAAAGA CATCATATTC TAAGTKGCAW ACGCATTCGC
1651 GTTAAATTTC ATTGCAGTCT TTATCTCACA TTATTCATAT TATGTATAAT
1701 CTTTATTTTG AATTTATATT TGACTTAACT TGATTAGTAT AAAACTAACT
1751 TTCGTTTACT TCAAAGTTTA AATCTTATCG AGTGATATTT CAGATTCTTT
1801 ATCTTTTTAT AAAATAGCCC TACAATTTAT AATTTTCCAC CCTAACTATA
1851 ATACTACAAA TAATAATTGG AATATATAGA TTTACTACTA AAGTATTAGA
1901 ACATTTCAAT AGAAGGTCGT TTCTTTCATA GTCATACGCA TTATATATAC
1951 CCTATTCTCA ATCTATTTAA TACGTAAAAC ATGAAATTTT CTTATTAAAT
2001 TTATTATTTC CATCATATCA TTACTTTTAA TTTAATGATG TTCAATTTAA

2051 ATATTAGGTC AATAACATAT TTATGCTTTT TATGGATACT TTCAAAAATA
2101 ACAGCCCCAA ACGATAACTT GAAAGGGGCT GTTAAATATT TAACTATTGC
2151 ATTTGATCKA TCATTYTMKW GKWTCYYYSR RTMMYKWKMT CRAAATACGT
2201 ATCGTATCTT TGCCATTCTT CTTGAGTAAT TGGCGTCATA TTTAATACAC
2251 CGCCAAGATC GACCTGCAGG CAT

Mutant: NT53

Phenotype: temperature sensitivity

Sequence map: Mutant NT53 is complemented by pMP143, which contains a 3.0 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 46, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to *papS*, encoding poly-A polymerase (EC 2.7.7.19) from *B. subtilis*(Genbank Accession No. L38424; published in Bower, S. *et al. J. Bacteriol.* 9 (1995) 2572-2575). Also included in this clone is the gene homolog for *birA*, which encodes biotin [acetyl-CoA-carboxylase] ligase and functions as a biotin operon repressor protein.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP143, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to augment the sequence contigs. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP143

SEQ ID NO. 41

pMP143.forward Length: 928 nt

1 TCCTCTAGAG TCGATCAATA TGAGTATTAT TATCAAAAAA TGCTAAATNA
51 GCATAACAAA AGTAAAGGCG AGTAATAATA TGGATAAATC ATTATTTGAA
101 YAGGCAAGGC CTATATTAGA ACAAATTCAA GACAATGGTT TTNAAGCATA
151 TTATGTAGGT GGCTCTGTAA GAGATTATGT CATGGGAAGA AATATTCATG
201 ATATAGATAT CACAACAAGT GCAACGNCGG ATGAAATAGA ATCTATCTTT
251 AGTCATACGA TACCTGTAGG TAAAGAACAT GGCACGATAA ATGTAGTTTT
301 TAATGATGAA AATTATGAAG TGACAACATT CCGGGCTGAA GAAGATTATG

351 TCGATCACCG TAGACCAAGT GGTGTTACAT TTGTYCGTGA TTTATACGAR
401 GATTTGCAAC GACGAGATTT CACGATGAAT GCGATAGAAT GGATACAGCA
451 TACAAATTGT ATGATTATTT TGATGGTCAA CAAGATATTA ATAATCGAWT
501 AATAAGAACT GTAGGTATAG CTGAGGAACG TTCCAAGAAG ATGCTTTACG
551 TATGATTCGA TGTTTAAGGT TCCAGTCACA ATTATCATTT GATATTGCAA
601 CGGAAACATT CGAAGCGATG CGTATACAAA TGGCAGATAT TAAATTTTTA
651 TCAATTGAGC GTATAGTGAT TGAACTAACT AAATTAATGC GAGGTATTAA
701 TGTTGAAAAG AGTTTTAATC ATTTAAAATC GCTGAAAGCA TTTAATTATA
751 TGCCGTATTT CGAACATCTT GATATGAATC AAATTAATGT AACTGAAGCA
801 ATTGATTTAG AATTGTTGAT TGCTATAGTA TCAGTTAAAT TTGATATTAA
851 TTACTCATTG AAGCCTTTAA AGCTAAGTTA ACCGACAAGT TAAAAGATAT
901 CAATCAATAT ATTCAAATTA TGAATGCA

SEQ ID NO. 42

pMP143.reverse Length: 2119 nt

1 TGCATGCCTG CAGGTCGATC TAATATAGTT TCCGCTAAAT ATAATTGTTG
51 CGGTCGATAT GTTAAGCCAR GTYGATCTAC AGCTTTGCTA TATAAAGACT
101 TCAAGCTGCC ATTATAATTT GTTGTCGGCT TTTTAAAATC AACTTGCTTA
151 CGATAGATAA TCTGTTCGAA CTTTTCGTAC GATTTATCCA ATGGCTTTGC
201 ATCATATTGC CTAACCATCT CAAAGAAAAT ATCATACAAA TCGTATTTCA
251 ACTGTTTACT TAAATAATAT AATTGCTTCA AAGTATCTAA CGGTAACTTT
301 TCAAATTTTT CAAAAGCTAA TATCATCAAT TTAGCAGTAG TAGCGGCATC
351 TTCGTCAGCT CGATGGGCAT TTGCTAAGGT AATACCATGT GCCTCTGCTA
401 ATTCACTTAA TTGATAGCTT TTATCTGTAG GAAAAGCTAT TTTAAAGATT
451 TCTAGTGTAT CTATAACTTT TTTGGGACGA TATTGAATAT TACAATCTTT
501 AAATGCCTTT TTAATAAAAT TCAAATCAAA ATCTACATTA TGAGCTACAA
551 AAATGCAATC TTTWATCTTA TCGTAGATTT CTTGTGCAAC TTGATTAAAA
601 TATGGCGCTT GTTGTAGCAT ATTTKCTTCA ATGGATGTTA ACGCWTGAAT
651 GAACGGCGGA AWCTCTAAAT TTGTTCTAAT CATAGAATGA TATGTATCAA
701 TAATTTGGTT ATTGCGSACA AACGTTATAC CAATTTGAAT GATATCGTCA
751 AAATCTAATT GGTTGCCTGT TGTTTCCAAA TCCACAACGG CATAGGTTGC
801 CATACCCATA GCTATCTCTC CTTGCTTTAG TGTTAAAAAT CTATATCTGC
851 ACTAATTAAA CGGTGTGATT CACCCGCTTC ATCTCTAACA ATTAGATAGC
901 CATCGTAATC TAAATCAATT GCTTGTCCTT TAAACTGTTT ATCATTTTCT
951 GTAAATAGCA ACGTTCTATT CCAAATATTA GAAGCTGCAG TATATTCTTC
1001 ACGAATTTCA GAAAAAGGTA ACGTTAAAAA TTGATTATAT CTTTTTYCAA
1051 TTTCTTGAAG TAATATCTCT AAAAATTGAT ATCTATCTAA TTWATTTTTA
1101 TCATGTAATT GTATACTTGT TGCTCTATGT CTAATACTTY CATCAAAGTT
1151 TTCTAGTTGT TTGCGTTCAA ATTAATACCT ATACCACATA TTATTGCTTC
1201 TATACCATCC ATTATTAGCA ACCATTTCAG TTAAGAAACC ACACACTTTA
1251 CCATTATCAA TAAATATATC ATTCGGCCAT TTCACTTTGA CTTCATCTTG
1301 ACTAAAATGT TGAATCGCAT CTCTTATCCC TAATGCAATA AATAAATTAA
1351 ATTTAGATAT CATTGAGAAT GCAACGTTAG GTCTTAACAC GACAGACATC
1401 CAAAGTCCTT GCCCTTTTGA AGAACTCCAA TGTCTATTAA ATCGCCCACG
1451 ACCTTTCGTT TGTTCATCAC TCAAGATAAA AAATGAAGAT TGATTTCCAA
1501 CAAGTGACTT TTTCGCAGCA AGTTGTGTAG AATCTATTGA ATCGTATACT
1551 TCACTAAAAT CAAACAAAGC AGAACTTTTT GTATATTGGT CTATTATACC
1601 TTGATACCAA ATATCTGGGA GCTGTTGTAA TAAATGCCCT TTATGATTTA
1651 CTGAATCTAT TTTACATCCC TCTAACTTTA ATTGGTCAAT CACTTTTTTT
1701 ACTGCAGTGC GTGGAAATAT TAAGTTGATT CCGCAATGCT TTGTCCAGAA

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1751  TATATAATTC GGTTTATTTT TATAGAGTAA TTGAAGTTAC ATCTTGACTA
1801  TATTTTNACA TGATTATCCA CCCATTTCAA AATTNCAGTT TCTNCGTTGC
1851  TTACTTTACC TGTNACAATC GCTATCTCAA TTTGTCTTAG CACATCTTTT
1901  AACCACGGAC CACTTTTGGC ATTTAAATGT GCCATAAGTA CACCGCCATT
1951  AACCATCATG TCTTTNCTAT TATGCATAGG TAAACGATGT AATGTTTCAT
2001  CAATCGTTTG AAGGTTAACG CTTAATGGTT CATGTCCTTG GTATCATAAC
2051  GCCTGTNTCA AGCGTTCTNC AANCATGTAC AGTTNTTCAA TGTGGNGTGT
2101  CCGNATTAAC GCTATTCAA
```

Mutant: NT54

Phenotype: temperature sensitivity

Sequence map: Mutant NT54 is complemented by pMP145, which contains a 3.1 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 47, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal identity at the nucleic acid level and peptide level to the C-terminal portion of the *pbp4* gene, encoding D,D-carboxy peptidase (EC 3.4.16.4) from *S. aureus*(Genbank Accession No. U29454; unpublished as of July, 1995). Since clone pMP146 does not contain the complete Pbp4 ORF, this gene is unlikely to be responsible for restoring mutant NT54 to a wild-type phenotype. Cross complementation with clone pMP91, which contains a 5.2 kb insert of *S. aureus* genomic DNA, reveals that only 800 additional base pairs downstream (3' to) the Pbp4 ORF are necessary for complementation (data not shown). DNA sequence of this region reveals strong similarity at the nucleic acid and peptide levels to the *tagD* gene, encoding glycerol-3-phosphate cytidylyl transferase (EC 2.7.7.39), from *B. subtilis* (Genbank Accession No. M57497; published in Mauel, C. *et al.*, *J. Gen. Microbiol.* 137 (1991) 929-941). The *tagD* gene of *B. subtilis* has been reported to be an essential gene and is therefore likely to be a good candidate for screen development. The relative size and location of the TagD ORF with respect to clone pMP145 is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence of the right-most portion of clone

pMP145, starting with the standard M13 reverse sequencing primer and applying primer walking strategies to complete the sequence contig. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP145
SEQ ID NO. 43

pMP145 Length: 1407 nt

```
   1 TTCACAGTGT TGTCGGGATA CGATATAGTA CACTGTACAG TACGNTGGAG
  51 ATTTATTAGA TTTTCACAGA ATTNTGAAAA TAAGACNACG GGTCATGGAA
 101 ATGTTACTAT TACCTGAACA AAGGCTATTA TATAGTGATA TGGTTGNTCG
 151 TATTTTATTC AATAATTCAT TAAAATATTA TATGAACGAA CACCCAGCAG
 201 TAACGCACAC GACAATTCAA CTCGTAAAAG ACTATATTAT GTCTATGCAG
 251 CATTCTGATT ATGTATCGCA AAACATGTTT GACATTATAA ATACAGTTGA
 301 ATTTATTGGT GAGAATTGGG ATAGAGAAAT ATACGAATTG TGGCGACCAA
 351 CATTAATTCA AGTGGGCATT AATAGGCCGA CTTATAAAAA ATTCTTGATA
 401 CAACTTAAAG GGAGAAAGTT TGCACATCGA ACAAAATCAA TGTTAAAACG
 451 ATAACGTGTA CATTGATGAC CATAAACTGC AATCCTATGA TGTGACAATA
 501 TGAGGAGGAT AACTTAATGA AACGTGTAAT AACATATGGC ACATATGACT
 551 TACTTCACTA TGGTCATATC GAATTGCTTC GTCGTGCAAG AGAGATGGGC
 601 GATTATTTAA TAGTAGCATT ATCAACAGAT GAATTTAATC AAATTAAACA
 651 TAAAAAATCT TATTATGATT ATGAACAACG AAAAATGATG CTTGAATCAA
 701 TACGCTATGT CRTATTTAGT CATTCCAGAA AAGGGCTGGG GACAAAAAGA
 751 AGACGATGTC GAAAAATTTG ATGTAGATGT TTTTGTTATG GGACATGACT
 801 GGGAAGGTGA ATTCGACTTC TTAAAGGATA AATGTGAAGT CATTTATTTA
 851 AAACGTACAG AAGGCATTTC GACGACTAAA ATCAAACAAG AATTATATGG
 901 TAAAGATGCT AAATAAATTA TATAGAACTA TCGATACTAA ACGATAAATT
 951 AACTTAGGTT ATTATAAAAT AAATATAAAA CGGACAAGTT TCGCAGCTTT
1001 ATAATGTGCA ACTTGTCCGT TTTTAGTATG TTTTATTTTC TTTTTCTAAA
1051 TAAACGATTG ATTATCATAT GAACAATAAG TGCTAATCCA GCGACAAGGC
1101 ATGTACCACC AATGATAGTG AATAATGGAT GTTCTTCCCA CATACTTTTA
1151 GCAACAGTAT TTGCCTTTTG AATAATTGGC TGATGAACTT CTACAGTTGG
1201 AGGTCCATAA TCTTTATTAA TAAATTCTCT TGGATAGTCC GCGTGTACTT
1251 TACCATCTTC GACTACAAGT TTATAATCTT TTTTACTAAA ATCACTTGGT
1301 AAAACATCGT AAAGATCATT TTCAACATAA TATTTCTTAC CATTTATCCT
1351 TTGCTCACCT TTAGACAATA TTTTTACATA TTTATACTGA TCAAATGAVC
1401 GTTCCAT
```

Mutant: NT55
Phenotype: temperature sensitivity
Sequence map: Mutant NT55 is complemented by pMP92, which contains a 2.0 kb insert of *S. aureus* genomic DNA. A

partial restriction map is depicted Fig. 48. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarity to the *nadE* gene product, encoding the nitrogen regulatory protein NH3-dependent NAD synthetase (EC 6.3.5.1), from *E. coli* (Genbank Accession No. M15328; published in Allibert, P. *et al.* *J. Bacteriol.* 169 (1987) 260-271).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP92, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP92
SEQ ID NO. 44

```
pMP92  Length: 1996 nt

   1  TCCTCTAGAG TCGATCGTAT TAAATTATCA AATAACGCTG AAAAGGTTAC
  51  GACGCCAGGT AAGAAAAATG TATATCGCAT TATAAACAAG AAAACAGGTA
 101  AGGCAGAAGG CGATTATATT ACTTTGGAAA ATGAAAATCC ATACGATGAA
 151  CAACCTTTAA AATTATTCCA TCCAGTGCAT ACTTATAAAA TGAAATTTAT
 201  AAAATCTTTC GAAGCCATTG ATTTGCATCA TAATATTTAT GAAAATGGTA
 251  AATTAGTATA TCAAATGCCA ACAGAAGATG AATCACGTGA ATATTTAGCA
 301  CTAGGATTAC AATCTATTTG GGATGAAAAT AAGCGTTTCC TGAATCCACA
 351  AGAATATCCA GTCGATTTAA GCAAGGCATG TTGGGATAAT AAACATAAAC
 401  GTATTTTTGA AGTTGCGGAA CACGTTAAGG AGATGGAAGA AGATAATGAG
 451  TAAATTACAA GACGTTATTG TACAAGAAAT GAAAGTGAAA AAGCGTATCG
 501  ATAGTGCTGA AGAAATTATG GAATTAAAGC AATTTATAAA AAATTATGTA
 551  CAATCACATT CATTTATAAA ATCTTTAGTG TTAGGTATTT CAGGAGGACA
 601  GGATTCTACA TTAGTTGGAA AACTAGTACA AATGTCTGTT AACGAATTAC
 651  GTGAAGAAGG CATTGATTGT ACGTTTATTG CAGTTAAATT ACCTTATGGA
 701  GTTCAAAAAG ATGCTGATGA AGTTGAGCAA GCTTTGCGAT TCATTGAACC
 751  AGATGAAATA GTAACAGTCA ATATTAAGCC TGCAGTTGAT CAAAGTGTGC
 801  AATCATTAAA AGAAGCCGGT ATTGTTCTTA CAGATTTCCA AAAAGGAAAT
 851  GAAAAAGCGC GTGAACGTAT GAAAGTACAA TTTTCAATTG CTTCAAACCG
 901  ACAAGGTATT GTAGTAGGAA CAGATCATTC AGCTGAAAAT ATAACTGGGT
 951  TTTATACGAA GTACGGTGAT GGTGCTGCAG ATATCGCACC TATATTTGGT
1001  TTGAATAAAC GACAAGGTCG TCAATTATTA GCGTATCTTG GTGCGCCAAA
1051  GGAATTATAT GAAAAAACGC CAACTGCTGA TTTAGAAGAT GATAAACCAC
1101  AGCTTCCAGA TGAAGATGCA TTAGGTGTAA CTTATGAGGC GATTGATAAT
1151  TATTTAGAAG GTAAGCCAGT TACGCCAGAA GAACAAAAAG TAATTGAAAA
1201  TCATTATATA CGAAATGCAC ACAAACGTGA ACTTGCATAT ACAAGATACA
1251  CGTGGCCAAA ATCCTAATTT AATTTTTTCT TCTAACGTGT GACTTAAATT
1301  AAATATGAGT TAGAATTAAT AACATTAAAC CACATTCAGC TAGACTACTT
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1351  CAGTGTATAA ATTGAAAGTG TATGAACTAA AGTAAGTATG TTCATTTGAG
1401  AATAAATTTT TATTTATGAC AAATTCGCTA TTTATTTATG AGAGTTTTCG
1451  TACTATATTA TATTAATATG CATTCATTAA GGTTAGGTTG AAGCAGTTTG
1501  GTATTTAAAG TGTAATTGAA AGAGAGTGGG GCGCCTTATG TCATTCGTAA
1551  CAGAAAATCC ATGGTTAATG GTACTAACTA TATTTATCAT TAACGTTTGT
1601  TATGTAACGT TTTTAACGAT GCGAACAATT TTAACGTTGA AAGGTTATCG
1651  TTATATTGCT GCATCAGTTA GTTTTTTAGA AGTATTAGTT TATATCGTTG
1701  GTTTAGGTTT GGTTATGTCT AATTTAGACC ATATTCAAAA TATTATTGCC
1751  TACGCATTTG GTTTTTCAAT AGGTATCATT GTTGGTATGA AAATAGAAGA
1801  AAAACTGGCA TTAGGTTATA CAGTTGTAAA TGTAACTTCA GCAGAATATG
1851  AGTTAGATTT ACCGAATGAA CTTCGAAATT TAGGATATGG CGTTACGCAC
1901  TATGCTGCGT TTGGTAGAGA TGGTAGTCGT ATGGTGATGC AAATTTTAAC
1951  ACCAAGAAAA TATGAACGTA AATTGATGGA TACGATAAAA AATTTA
```

Mutant: NT57

Phenotype: temperature sensitivity

Sequence map: Mutant NT57 is complemented by pMP94, which contains a 3.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 49, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal significant similarity at the peptide level to the *gap* gene, encoding glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), from a number of prokaryotes and eukaryotes (e.g. Genbank Accession No. M24493, for the corresponding gene from *B. stearothermophilus*; published in Branlandt, C. *et al.*, 1989, *Gene* 75:145-155). From the opposite sequence contig, a strong peptide-level similarity is noted to the dnaB gene product, encoding an essential protein involved in the initiation of DNA replication, from *B. subtilis* (Genbank Accession No. M15183; published in Hoshino, T. *et al.* *Proc. Natl. Acad. Sci. USA* **84** (1987) 653 - 657). Also of significance is the similarity of a subclone sequence to an ORF of unknown function, conserved among prokaryotes including *E. coli*, *M. leprae*, *C. acetobutylicum*, *H. influenzae* and *B. subtilis* (e.g. "orf 168" from Genbank Accession No. D28752). The relative orientations and predicted sizes of the ORFs identified in this entry are denoted by arrows in the restriction map.

DNA sequence data: The following DNA sequence data represents the partial sequence of clone pMP94, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to augment the sequence contigs as well as obtain subclone sequence data. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP94

SEQ ID NO. 45
pMP94.forward Length: 1017 nt

```
   1  CTTYGARCTC GGTACCCGGG GMTCCTCTAR AGTCGATCTT TATACTCTTG
  51  TAACACATTT AAGTCTTCAT CAATCATAGC ATTCGTTAAT TCAGCTCGAT
 101  GCGCTTCCAA AAATTGCTTA ACATCTGGGT CATWGATGTC TCCTGATTTT
 151  ATCTTTTCTA TTCTTTTTTC AAAGTCCTGC GACGTGTTAA TTATACTTTT
 201  AAATTGCTTC ATTATTGACT GTCCTCCTCC CATTTTTTAG ATAATTTATC
 251  TAGAAATGCT TGTCGATCTT GCTCTAATTG TTGATCATCT ACGCTATTAT
 301  CTTTAGCCGA ATCTTCTTCA CTAGGTTTAT CTCTATTTTC TAACCATTTA
 351  GGTGTTTTTT CTTTTGAAAT ACGATTACGC TGCCCATAGT ATGAACCACG
 401  CTTTTGGTAA TTTCCGCTAG AACCCTCATT TTTAGGTTGA TTAACTTTTT
 451  TAGCGTAATT ATATGCTTCT TTAGCTGTCT TAATACCTTT TTTCTTCCAA
 501  TTTGATGCTA TTTCCAAAAT ATACGCTTTA GGAAGTTTCA TATCTTCTTT
 551  TAACATGACA AATTGCAACA AAATATTAAT GACGCCAAAA GACATTTTTT
 601  CACGTTTCAA TTAATTCTTC AACCATTGTC TTTTGCGATA TAGTTGGTYC
 651  TGATTCAGAM CAAGAAGCTA ACATATCAAT TGGACTCGTT TGTTCAAGTA
 701  ACTCAAACCA TTCATCACTT TGTGGCTTTG GATTCACTTC TGAAGATTTG
 751  CCCGCCGAAG ATGATGTAGC AGGAGATTTC ACCTGTAATT TAGGCATTTG
 801  ATTTTCGTGT TCCATTAAGT AATACGAGCG TGCTTGTTTA CGCATTTCTT
 851  CAAAGGATAA CTGTTGTCCA CTTGTAATTG AATTTAAAAT AACATGCTTC
 901  ATGCCATCTG CTGTTAAACC ATATAAATCN CGAATTGTGT TATTAAACCC
 951  TTGCATCTTG GTAACAATGT CTTGACTAAT AAATGTTTAC CTAACATTGT
1001  CTCCACATTT CNANTCC
```

SEQ ID NO. 46
pMP94.reverse Length: 1035 nt

```
   1  TGCATGCCTG CAGGTCGATC AAGGGGTGCT TTTAATGTCA AMGAATATTG
  51  CAATTRATGG TATGGGTAGA ATTGGAAGAA TGGTATTACG TATTGCATTA
 101  CAAAATAAAA ATTTAAATGT AGTAGCGATA AATGCTAGTT ATCCACCCGA
 151  AACAATTGCA CATTTAATCA ATTACGATAC GACACATGGA AAATATAATC
 201  TAAAAGTTGA ACCGATTGAA AATGGATTGC AAGTTGGAGA TCATAAAATT
 251  AAATTGGTTG CTGATCGCAA TCCTGAAAAC TTGCCATGGA AAGAATTAGA
 301  TATCGATATT GCTATAGATG CAACTGGTAA ATTTAATCAT GGTGATAAAG
 351  CCATCGCACA TATTAAAGCA GGTGCCAAAA AAGTTTTGTT AACTGGTCCT
 401  TCAAAAGGTG GACATGTTCA AATGGTAGTT AAAGGCGTAA ATGATAACCA
 451  ATTAGATATA GAAGCATTTG ACATTTTTAG TAATGCTTCA TGTACTACTA
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501 ATTGCATTGG TCCAGTTGCA AAAGTTTTAA ATAATCAGTT TGGGAATAGT
551 TAATGGTTTA ATGACTACTG TTCACGCTAT TACAAATGAC CAAAAAAATA
601 TTGATAATCC MCATAAAGAT TTAAGACGTG CACGTTCATG TWATGAAAGC
651 ATTATTCCTA CTTCTACTGG TGCGGCGAAA GCTTTAAAAG AAGTATTACC
701 AGAATTAGAA GGTAAATTAC ACGGCATGGC ATTACGTTGT ACCAACAAAG
751 AATGTATCGC TCGTTGATTT AGTTGTTGAT TTAGAAAAAG AAGTAACTGC
801 AGAAGAANTA AACCAAGCTT TTGAAAATGC AGGTTTAGAA GGTATCATAG
851 AANTCGAACA TCACCACTAG TGTCTGTTGA TTTTAATACT AATCCCAATT
901 CAGCTATTAT TGATGCCAAA CCACNATGTC ATGTTCCGGG AAATAAGTAA
951 ANTTATTGCT TGGTATGAAN ATGAATGGGG TTATTCCAAT AAATTGTTAA
1001 NNTTGCNGAA CAAATTGGAC NCTTTGGANT CCAAA

SEQ ID NO. 47

pMP94.subclone Length: 483 nt

1 CTCCGTTTGT TTTCGCTTAA AATCCCTTGC ATCGATGCTA ACAATTGATC
51 AACATCTTTA AATTCTTTAT AGACTGATGC AAATCTAACA TATGAAACTT
101 GATCAACATG CATTAACAAG TTCATAACGT GTTCACCTAT ATCTCGTGAA
151 GACACTTCCG TATGACCTTC ATCTCGTAAT TGCCATTCAA CCTTGTTAGT
201 TATGACTTCA AGTTGTTGAT ATCTAACTGG TCGTTTCTCA CAAGAACGCA
251 CAAGTCCATT AAGTTATCTT TTCTCTTGAA AACTGCTCTC TTGTGCCATC
301 TTTTTTCACA ACTATAAGCT GACTAACTTC GATATGNTTC AAATGTTAGT
351 GGAAACGTTG TTTCCACAAT TTTCACATTC TCTTCGTCTT CCGAAATGGC
401 ATTTAATTCA TCGGGCATGC CTTGAATCTA CAACTTTAGA ATTGTGTTAG
451 AATTACATTT CGGGCATTTC ATTACATCAC CTC

Mutant: NT68
Phenotype: temperature sensitivity
Sequence map: Mutant NT68 is complemented by pMP163, which contains a 5.8 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 50. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to the *dnaE* gene, encoding DNA polymerase III alpha subunit (EC 2.7.7.7), from Gram-negative bacteria such as *S. typhimurium* (Genbank Accession No. M29701; published in Lancey, E.D., *et al. J. Bacteriol.* **171** (1989) 5581 - 5586). This mutant is distinct from NT28, described previously as having a mutation in the *polC* gene which also encodes an alpha subunit of DNA polymerase III (found so far in Gram-positive bacteria). Although *dnaE* and *polC* putatively encode proteins of the same enzymatic function, in *S. aureus* these two genes are quite distinct and may or may not encode proteins of redundant function; since the DNA

sequences of each are less than 65% identical, they are confirmed as being two distinct essential genes.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP163, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP163
SEQ ID NO. 48

pMP163 Length: 5718 nt

```
   1 CTCGGTACCC GGGGATCGTC ATGGAATACC GGAATATTAG TTTCTTTTTT
  51 CAATCGTTCT TCAATTTCAA AACAACGTGG TGCCGAAATA TCCTCTAAAT
 101 TAATACCACC ATAATTAGGT TCTAACAACT TAACTGTTTT AATGATTTCT
 151 TCGGTATCAG TTGTATTTAA CGCAATAGGC ACCCCATTGA TACCAGCGAA
 201 GCTTTTGAAT AATACTGCTT TACCTTCCAT TACAGGAATA CTTGCTTCAG
 251 GTCCAATGTT ACCTAAACCT AATACCGCTG TTCCATCAGT AATAACTGCA
 301 ACTGTATTTC CTTTAATTGT GTAATCATAT ACTTTTCTTT TATCTTCATA
 351 AATATCTTTA CACGGTTCAG CAACGCCAGG TGAGTATGCT AAACTTAATT
 401 CCTCTTTATT AGTAACTTTT ACATTTGGTT TAACTTCTAA TTTACCTTGA
 451 TTACGTTTGT GCATTTCCAA TGCTTCATCT CTTAATGACA TGAAATCAGC
 501 CCCTAATTCA ATATTTATTT TTAAAAAATA ACTTGGATAA AACGCATTAC
 551 ATTATAAAAG TAAAAATATT GGGTAATCTG AATGARTAAG AATTTATGGT
 601 TTTGATTATG TAACACAAAT AGCGATAAAC GATAATAAAA TAATATTTAT
 651 AAAGATACAT TAAACCATAC TATCTAAAGA TATACCTTTA ATTATTATAA
 701 TGGATAGCAA AAACCAATAT ATCAAAAAGT TATTATTTTT CCGCACGATA
 751 TATCGACAAA ATTCTTTACT CAATTTATGT ATACTGCTTT TTGTGCTAAT
 801 TATTCTTATG GATTAATCAA TAATGTAAAG TGAAACTCAT AAAAATAATA
 851 AGCATAAAAA ACTAATATAA ACGCAAACTG ATGGTTAAAA AATATCTAAC
 901 CATCAGTTTA CTATATCATA ATTTATTAGT TGATAAAAGT TATATAAGCC
 951 TAATATCACT AGGGTTAAAG GGATTGTATA AAATTATTAA ACATACTATC
1001 TTTTTGATTA ATATAGCCTA AAGTAGTCAT TTGTTTAATC GTTTCATCAT
1051 AAAAGGATAA CACAACATCA TTAGCATTCT CTTTCGTAGC TTTAATCATC
1101 TCTTCAAACA TATCTATTTG TGATTTATTT CTAATTATAA TTTGTTTGGC
1151 AAATGCTAAT TTTTGTTCTT CAAAAGTGGC TAATGTCTGA ATCTCATTTA
1201 TAATTAGTTG ACGTTGTTGC TTTCTATGGT CAAATTTCCC GCTAACTATA
1251 AACAAGTCAT TATGTGATAA CAACTCTTCG TACTTTTTAA ACTGATTAGG
1301 GAAAATCACA CCATCTAAAG TTTCAATGCC ATCATTTAAT GTTGACGAAT
1351 GCCATATTTT GACCATTTTT AGTTCGAATT TGTTTAACTT TATCAAACTG
1401 TACTAATATA GGTTTATAAT TCTGCGCGTT ACTCAATTTA AATATCGTTA
1451 AATATTGTTT GGCAACAAAC TTTTTATCTA CTGGGTGTTG CGAAACATAA
1501 AATCCTAAAT ATTCTTTTTC GTACTGACTA ATAAGTGCAT CAGGCAATTC
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1551 TTCTTTATCT TCATACATCT GTTTTGGCGT TAAAATATCA AATAAAAAAC
1601 CATCTTGTTC AATGTTTAAA TCGCCATCCA ACACTTGATC AATAGCTTGC
1651 AACAACGTTG AACGTGTTTT ACCAAAAGCA TCAAACGCTC CCACTAAAAT
1701 CAGTGCTTCA AGTAACTTTC TCGTTWTGAM YCTCTTCGGT ATACGTCTAG
1751 CAWAATCAAA GAAATCTTTA AATTTGCCGT TCTGATAACG TTCATCAACA
1801 ATCACTTTCA CACTTTGATA ACCAACACCT TTAATTGTAC CAATTGATAA
1851 ATAAATGCCT TCTTGGGAAG GTTTATAAAA CCAATGACTT TCGTTAATGT
1901 TCGGTGGCAA TATAGTGATA CCTTGTTTTT TTGCTTCTTC TATCATTTGA
1951 GCAGTTTTCT TCTCACTTCC AATAACATTA CTTAAAATAT TTGCGTAAAA
2001 ATAATTTGGA TAATGGACTT TTAAAAAGCT CATAATGTAT GCAATTTTAG
2051 AATAGCTGAC AGCATGTGCT CTAGGAAAAC CATAATCAGC AAATTTCAGA
2101 ATCAAATCAA ATATTTGCTT ACTAATGTCT TCGTGATAAC CATTTTGCTT
2151 TGSMCCTTCT ATAAAATGTT GACGCTCACT TTCAAGAACA GCTCTATTTT
2201 TTTTACTCAT TGCTCTTCTT AAAATATCCG CTTCACCATA ACTGAAGTTT
2251 GCAAATGTGC TCGCTATTTG CATAATTTGC TCTTGATAAA TAATAACACC
2301 GTAAGTATTT TTTAATATAG GTTCTAAATG CGGATGTAAA TATTGAACTT
2351 TGCTTGGATC ATGTCTTCTT GTAATGTAAG TTGGAATTTC TTCCATTGGA
2401 CCTGGTCTAT ACAAAGAAGT TACAGCAACA ATATCTTCAA AGTGTTCCGG
2451 CTTTAATTTT TTTAATACAC TTCTTACACC GTCAGACTCT AATTGGAATA
2501 TGCCAGTCGT ATCTCCTTGC GACAACAATT CAAACACTTT TTGATCATCA
2551 AACGGAATCT TTTCGATATC AATATTAATA CCTAAATCTT TTTTGACTTG
2601 TGTTAAGATT TGATGAATAA TCGATAAGTT TCTCAACCCT AGAAAATCTA
2651 TTTTTAATAA CCCAATACGT YCGGCTTCAG TCATTGTCCA TTGCGTTAAT
2701 AATCCTGTAT CCCCTTTCGT TAAAGGGGCA TATTCATATA ATGGATGGTC
2751 ATTAATAATA ATYCCTGCCG CATGTGTAGA TGTATGTCTT GGTAAACCTT
2801 CTAACTTTTT ACAAATACTG AACCAGCGTT CATGTCGATG GTTTCGATGT
2851 ACAAACTCTT TAAAATCGTC AATTTGATAT GCTTCATCAA GTGTAATTCC
2901 TAATTTATGT GGGATTAAAC TTGAAAATTT CATTTAATGT AACTTCATCA
2951 AACCCCATAA TTCTTCCAAC ATCTCTAGCA ACTGCTCTTG CAAGCAGATG
3001 AMCGAAAGTC ACAATTCCAG ATACATGTAG CTCGCCATAT TTTTCTTGGA
3051 CGTACTGAAT GACCCTTTCT CGGCGTGTAT CTTCAAAGTC AATATCAATA
3101 TCAGGCATTG TTACACKTTC TGGGTTTAAA AAACGTTCAA ATAATAGATT
3151 GAATTTAATA GGATCAATCG TTGTAATTCC CAATAAATAA CTGACCAGTG
3201 AGCCAGCTGA AGAACCACGA CCAGGACCTA CCATCACATC ATTCGTTTTC
3251 GCATAATGGA TTAAATCACT WACTATTAAG AAATAATCTT CAAAACCCAT
3301 ATTAGTAATA ACTTTATACT CATATTTCAA TCGCTCTAAA TAGACGTCAT
3351 AATTAAGTTC TAATTTTTTC AATTGTGTAA CTAAGACACG CCACAAATAT
3401 TTTTTAGCTG ATTCATCATT AGGTGTCTCA TATTGAGGAA GTAGAGATTG
3451 ATGATATTTT AATTCTGCAT CACACTTTTG AGCTATAACA TCAACCTGCG
3501 TTAAATATTT CTTGGTTAAT ATCTAATTGA TTAATTTCCT TTTTCAGTTA
3551 AAAAATGTGC ACCAAAATCT TTCTTGATCA TGAATTAAGT CTAATTTTGT
3601 ATTGTCTCTA ATAGCTGCTA ATGCAGAAAT CGTATCGGCA TCTTGACGTG
3651 TTTGGTAACA AACATTTTGA ATCCAAACAT GTTTTCTACC TTGAATCGAA
3701 ATACTAAGGT GGTCCATATA TGTGTCATTA TGGGTTTCAA ACACTTGTAC
3751 AATATCACGA TGTTGATCAC CGACTTTTTT AAAAATGATA ATCATATTGT
3801 TAGAAAATCG TTTTAATAAT TCAAACGACA CATGTTCTAA TGCATTCATT
3851 TTTATTTCCG ATGATAGTTG ATACAAATCT TTTAATCCAT CATTATTTTT
3901 AGCTAGAACA ACTGTTTCGA CTGTATTTAA TCCATTTGTC ACATATATTG
3951 TCATACCAAA AATCGGTTTA ATGTTATTTG CTATACATGC ATCATAAAAT
4001 TTAGGAAAAC CATACAATAC ATTGGTGTCA GTTATGGCAA GTGCATCAAC
4051 ATTTTCAGAC ACAGCAAGTC TTACGGCATC TTCTATTTTT AAGCTTGAAT
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4101  TTAACAAATC ATAAGCCGTA TGAATATTTA AATATGCCAC CATGATTGAA
4151  TGGCCCCTTT CTATTAGTTA AGTTTTGTGC GTAAAGCTGT AGCAAGTTGC
4201  TCAAATTCAT CCCAGCTGTC CAACTGAAAY TCCTGACGCA TTCGGATGAC
4251  CACCGCCACC AAAATCTTGC GCAATATCAT TAATAATCAA TTGCCCTTTA
4301  GAACGTAATC GACATCTGAT TTCATTACCT TCATCGACTG CAAATACCCA
4351  TATTTTCAAG CCTTTGATGT CAGCAATTGT ATTAACAAAC TGAGATGCTT
4401  CATTTGGCTG AATACCGAAT TGCTCCAATA CATCTTCAGT TATTTTAACT
4451  KGGCAGAATC CATCATCCAT AAGTTCGAAA TGTTGYAAAA CATAACCTTG
4501  AAACGGCAAC ATTKYTGGGT CCTTCTCCAT CATTTTATTT AAAAGCGCAT
4551  TATGATCAAT ATCATGCCCA ATTAACTTTC CAGCAATTTC CATAGTATGT
4601  TCWGAGGTAT TGTTAAAAAG GRGATCGCCC AGTATCACCG ACGATACCAA
4651  GATATAAAAC GCTCGCGATA TCTTTATTAA CAATTGCTTC ATCATTAAAA
4701  TGTGAGATTA AATCGTAAAT GATTTCACTT GTAGATGACG CGTTCGTATT
4751  AACTAAATTA ATATCACCAT ACTGATCAAC TGCAGGATGA TGATCTATTT
4801  TAATAAGTYT ACGACCTGTA CTATAACGTT CATCGTCAAT TCGTGGAGCA
4851  TTGGCAGTAT CACATACAAT TACAAGCGCA TCTTGATATG TTTTATCATC
4901  AATGTTATCT AACTCTCCAA TAAAACTTAA TGATGATTCC GCTTCACCCA
4951  CTGCAAATAC TTGCTTTTGC GGAAATTTCT GCTGAATATA GTATTTTAAA
5001  CCAAGTTGTG AACCATATGC ATCAGGATCK RSTYTARMRK RTCYSYGKMT
5051  AMYRATTGYA TCGTTGTCTT CGATACATTT CATAATTTCA TTCAAAGTAC
5101  TAATCATTTT CAWACTCCCT TTTTTAGAAA AGTGGCTTAA TTTAAGCATT
5151  AGTCTATATC AAAATATCTA AATTATAAAA ATTGTTACTA CCATATTAAA
5201  CTATTTGCCC GTTTTAATTA TTTAGATATA TATATTTTCA TACTATTTAG
5251  TTCAGGGGCC CCAACACAGA GAAATTGGAC CCCTAATTTC TACAAACAAT
5301  GCAAGTTGGG GTGGGGCCCC AACGTTTGTG CGAAATCTAT CTTATGCCTA
5351  TTTTCTCTGC TAAGTTCCTA TACTTCGTCA AACATTTGGC ATATCACGAG
5401  AGCGCTCGCT ACTTTGTCGT TTTGACTATG CATGTTCACT TCTATTTTGG
5451  CGAAGTTTCT TCCGACGTCT AGTATGCCAA AGCGCACTGT TATATGTGAT
5501  TCAATAGGTA CTGTTTTAAT ATACACGATA TTTAAGTTCT CTATCATGAC
5551  ATTACCTTTT TTAAATTTAC GCATTTCATA TTGTATTGTT TCTTCTATAA
5601  TACTTACAAA TGCCGCTTTA CTTACTGTTC CGTAATGATT GATTAAAAGT
5651  GGTGAAACTT CTACTGTAAT TCCATCTTGA TTCATTGTTA TATATTTGGC
5701  GATTTGATCC TCTAGAGT
```

Mutant: NT78

Phenotype: temperature sensitivity

Sequence map: Mutant NT78 is complemented by pMP115, which contains a 5.3 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 51, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal no significant similarities between the sequences obtained at the left-most and right-most edges and any published sequences. The sequence generated from a Msp I subclone,

however, matches at both the nucleic acid and peptide level to *hsp60*, encoding the GroEL protein from *S. aureus* (Genbank Accession No. D14711). The relative size and orientation of the GroEL ORF is depicted by an arrow; other proteins (i.e. GroES) are known to reside near the identified ORF and will be confirmed by further DNA sequencing.

DNA sequence data: The following DNA sequence data represents the sequence generated bye sequencing the left-most and rightmost edges of pMP115 and its subclone 78.3, starting with standard M13 forward and M13 reverse sequencing primers. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP115, a 5,300 bp genomic fragment

SEQ ID NO. 49

pMP115.m13f Length: 513 nt

```
  1  TTCTTGCCTC CCAATCGCCT AATAGCCCTN AAAACTACTT TTTTTAATCT
 51  ATAGGCGATG TAAAAATACC ATATATTGAN GGTGCTATAC CTCCTAAAAT
101  AGCAGTTCCC AAAGTTGTCA TTACTGAAAT TACTGCGAAA GTATCATCCG
151  AAAGCAATAA ATTCAAACTA ATGCATTGTT TATTACCCAT CGAATTTATT
201  GACCAAATAG CTAGAGAAAT AAACAACCCA AAATTTAAAA TAAATGATAT
251  AGTAATAGCA ATTGTTTACA AAACACGGAA TTTTTCATTT TTATTTATAT
301  TATCCATTTT NCTCCCTTTT NCTTAAATCA TTTTATTATA TATTNCAATA
351  ATCAATCTGA AATGTTGATG TAATTTGNNA AAAATATCAT ACTTTTNCTC
401  CTGAAAACCT CCCTAAATCA TCAATATGGN AATCNGTNTT NGGGTATTGC
451  GNTTNCAACT CTTTTAAANC TCACTCNTTC TTCTCATCGN CTTAACCGTA
501  CTATCANTAA AAT
```

SEQ ID NO. 50

pMP115.m13r Length: 533 nt

```
  1  CTGAGCTGCT TNCANNNCCA NTNTGAAAAA GCCCCCAGNN CAGCCCGNTT
 51  NCAAAACAAC GNCTNCATTT GAANCCCCAT GAAAAAGAAC GAATTTTGAC
101  AATGGNTTAA AAAACANGNA AGATAATAAG AAAAAGTGCC GTCAACTGCA
151  TATAGTAAAA GTTGGCTAGC AATTGTATGT NCTATGATGG TGGTATTTTC
201  AATCATGCTA TTCTTATTTG TAAAGCGAAA TAAAAAGAAA AATAAAAACG
251  AATCACAGCG ACGNTAATCC GTGTGTGAAT TCGTTTTTTT TATTATGGAA
301  TAAAAATGTG ATATATAAAA TTCGCTTGTC CCGTGGCTTT TTTCAAAGCC
351  TCAGGNTTAA GTAATTGGAA TATAACGNCA AATCCGTTTT GTAACATATG
401  GGTAATAATT GGGAACAGCA AGCCGTTTTG TCCAAACCAT ATGCTAATGN
451  AAAAATGNCA CCCATACCAA AATAAACTGG GATAAATTTG GNATCCATTA
501  TGTGCCTAAT GCAAATNCCT NATGACCTTC CTT
```

The following DNA sequence data were acquired using standard sequencing methods and the commercially-available T7 and SP6 primers and can be used to demonstrate identity to the GroEL protein from S. aureus:

subclone 78.3, a 2000 bp Msp I fragment

SEQ ID NO. 51

78.3.sp6 Length: 568 nt

```
  1 CCGACAGTCG TTCCCNTCAT GCAAAATATG GGGGCTAAAC TCAGTTCAAG
 51 AAGTCGGCAA ATAAGACAAA TGAAATTGCC TGGTGACGGT AGNACAACTG
101 CAACAGTATT AGCTCAAGCA ATGATTCAAG AAGGCTTGAA AAATGTTACA
151 AGTGGTGCGA ACCCAGTTGG TTTACGACAA GGTATCGACA AAGCAGTTAA
201 AGTTGCTGTT GAAGCGTTAC ATGAAAATTC TCAAAAAGTT GAAAATAAAA
251 ATGAAATTNC GCAAGTAGGT GCGNTTTCAG CAGCAGATGN AGNAATTNGA
301 CGTTATATTT CTGAAGCTAT NGGNAAAGTA GGTAACGNTG GTGTCATTAC
351 ANTTNTNGGG TCAAATGGGC TNTNCACTNN NCTNGANGTG GTTGNNGGTG
401 TNCNATTTGA TCNNNGTTAT CANTCACCNN CTATNGTTAC TGCTTCNGCT
451 AAAATGGTTG CTGCNTTTGG NCGCCCCTAC ATTTTTGTNA CNGCTTNGGG
501 ANTCTCGTCT TTNCNCGATT CTTTCCCCTT TTTGGCCCNT GGGNAATCTT
551 TTNGGNCNCC CTTTATTT
```

Mutant: NT81

Phenotype: temperature sensitivity

Sequence map: Mutant NT81 is complemented by clone 81-3, which contains a 1.7 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 52, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity to the *fib* locus, encoding a fibrinogen binding protein, from *S. aureus* (Genbank Accession No. X72013; published in Boden, M.K. *et al.*, *Mol. Microbiol*. 12 (1994) 599-606.) The relative size and orientation of the Fib ORF with respect to the restriction map is depicted by an arrow; also identified in this analysis is an ORF of unknown function downstream from (3' to) the Fib ORF.

DNA sequence data:. The following DNA sequence data represent the sequences at the left-most and right-most edges of subclones pMP1043 and pMP1042, using standard SP6 and T7 sequencing primers. The sequences below can be used

to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

subclone 1042, a 400 bp Hind III fragment

SEQ ID NO. 52

1042.con Length: 437 nt

```
  1 CAAYTTAGYC AACTACTACC AATATAGCAC TAGAACTGGA AATGATAATT
 51 TAATATTGKG CACTTTTTSA TTGKTTAAAC ATGTACATAT TTNAAAAAAT
101 AGGAGAGCAA AGKAAATAAT TGATATAGTT ATTTTSAGAG TAATCCTAGG
151 AACTATTGTA TTTATATTTS TCTCCCCTAC TTTTAAATGT CATTCATTAT
201 ACATAAGCAT TTTGATATAG AATTTATCAC ATATGCAAAT TGAAAACAGG
251 TTAAGACCAT TTTTTGTCTC AACCTGTTTT ATTTATTATC TATTTMTAAT
301 TTCATCAATT TCTTTGTATA TTTTTYCTAA TGCAACTTTA GCATCAGCCA
351 TTGATACGAA ATCATTTTYC TTAAGTGCCG CTTTAGCTCT ATATTCATTC
401 ATYATAATCG TACGTTTATA ATATGGATTT ACGTTGA
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subclone 1043, a 1300 bp EcoR I/ Hind III fragment

SEQ ID NO. 53

1043.t7 Length: 659 nt

```
  1 CCCGATTCGA GCTCGGTACC GGNGATCCTC TAGAGTCGAT CTATCAAGCA
 51 GTAAATGAAA AAATGGACAT TAATGATATT AATATCGACA ATTTCCAATC
101 TGTCTTTTTT GACGTGTCTA ATTTGAATTT AGTAATTCTA CCAACGTTAA
151 TCATTAGCTG GGTCACAATA TTTAACTATA GAATGAGAAG TTACAAATAA
201 AATCTATGAG ATTATACCTN CAGACACCAA CATTCAAATG GTGTCTTTTN
251 TGTTGTGTGG TTTTATTTNT GAAATNCGAA AAAGTAGAGG CATGAATTTT
301 GTGACTAGTG TATAAGTGCT GATGAGTCAC AAGATAGATA GCTATATTTT
351 GTCTATATTA TAAAGTGTTT ATAGNTAATT AATAATTAGT TAATTTCAAA
401 AGTTGTATAA ATAGGATAAC TTAATAAATG TAAGATAATA ATTTGGAGGA
451 TAATTAACAT GAAAAATAAA TTGATAGCAA AATCTTNATT AACATTAGGG
501 GCAATAGGTA TTACTACAAC TACAATTGCG TCAACAGCAG ATGCGAGCGA
551 AGGATACGGT CCAAGAGAAA AGAAACCAGT GAGTATTAAT CACAATATCG
601 NAGAGTACAA TGATGGTACT TTTAATATCA ATCTTGANCA AAATTACTCA
651 ACAACCTAA
```

SEQ ID NO. 54

1043.sp6 Length: 298 nt

```
  1 AATNCTCCTC CNATGNTTTA TNATGAAACT AACTTTAAGT NAAATATTTN
 51 TCCAGACTAC TTGCATCTCC NTTATNCCCT TCTATAGTTN CTATCCCAGT
101 TNATGATAAA AGTAATGCTA ATGTNCCTGT NAATATATAT TTNTAAAATT
151 NNATTATAAG CNCTCCTTAA AATTNATACT TACTGAGTAT ATAGTCAATT
201 TNNGGACAAT TACATTAACC TGTCATTAAA TNGATTACTT TTTNNATTAA
251 CAAAAATTAA CATAACATTT AATTAATTNT TTCCNGATAN CAGCAACG
```

Mutant: NT86

Phenotype: temperature sensitivity

Sequence map: Mutant NT86 is complemented by pMP121, which contains a 3.4 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 53, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity at the nucleic and peptide levels to the *dnaK/dnaJ* genes, encoding Hsp70 and Hsp40, from *S. aureus* (Genbank Accession No. D30690; published in Ohta, T. *et al. J. Bacteriol.* 176 (1994) 4779-4783). Cross complementation studies (plasmid pMP120; data not shown) reveal that the ORF responsible for restoring a wild-type phenotype to mutant NT86 codes for Hsp40. The relative sizes and orientations of the identified genes are depicted in the restriction map by arrows.

DNA sequence data: The following DNA sequence data represent the sequences at the left-most and right-most edges of clone pM121, using standard M13 forward and M13 reverse sequencing primers. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP121, a 3400 bp genomic fragment

SEQ ID NO. 55

pMP121.m13f Length: 535 nt

```
  1 TCCAAATATT CACCAAGCTG TAGTTCAAGA TGATAACCCT NATTTTAANT
 51 CTGGCGAAAT CACTCAAGAN CTACAAAAAG GATACAAGCT TAAAGATAGA
101 GTATTAAGAC CATCANTGGT CAAAGTAAAC CAATAACTTA AATTTGGCGA
151 AAAGACATTG TTTAAAATTA ANTTAATTTA ATGATTAATT GGAGGNATTT
201 TNTTATGAGT AAAATTNTTG GTATAGACTT AGGTACAACA NATTCATGTG
251 TAACAGTATT AGANGGCGAT GAGCCAAAAG TAATTCAAAA CCCTGANGGT
301 TCACGTACAA CACCATCTGT NGTAGCTTTC AAAAATGGAG AAACTCAAGT
351 TGGTGAAGTA GCAAAACGTC AAGCTATTAC AAACCCAAAC ACTGTTCANT
401 CTATTAGNCG TCATATGGGT ACTGNTTATA ANGTAGATAT TGAGGGTAAA
451 TCATACACAC CACAAGNNNT CTCAGCTNTG NTTTTNCAAA ACTTANNANT
501 TNCAGCTGNA GTNATTTAGG TGNGNNNGTT GNCAA
```

SEQ ID NO. 56

pMP121.m13r Length: 540 nt

```
  1 ATGACTGCAG GTCGATCCAT GATTTACAAG TATATTGGTA GCCAATTCTA
```

51 CTGCTTCATG ATTAATAATA ATTGAAAGCT CTGTCCAGTT CATACTTTAT
101 TCTCCCTTAA AGAATCTTTT TGNTCTATCT TTAAAATTCG AAGGTTGTTC
151 ATTAATTTCT TCACCATTTA ATTGGGCAAA TTCTTTCATT AGTTCTTTNT
201 GTCTATCTGT TAATTTAGTA GGCGTTACTA CTTTAATATC AACATATAAA
251 TCTCCGTATC CATAGCCATG AACATTTTTT ATACCCTTTT CTTTTAAGCG
301 GAATTGCTTA CCTGTTTGTG TACCAGCAGG GGATTGTTAA CATAACTTCA
351 TTATTTAATG TTGGTATTTT TATTTCATCG CCTAAAGCTG CTTGTGGGAA
401 GCTAACATTT AATTTGNAAT AAATATCATC ACCATCACGT TTAAATGTTT
451 CAGATGGTTT AACTCTAAAT ACTACGTATT AATCANCAGG AGGTCCTCCA
501 TTCACGGCTG GAGAGGCTTC AACAGCTAAT CTTATTTGGT

The following DNA sequence data were acquired using standard sequencing methods and the commercially-available T7 and SP6 primers and can be used to demonstrate identity to the Hsp40 protein from *S. aureus*.

subclone 1116, a 1400 bp EcoR I/ Hind III fragment

SEQ ID NO. 57
1116.sp6 Length: 536 nt
1 TTTATAATTT CATCTNTTGA AGCATCCTTA CTAATGCCTA AAACTTCATA
51 ATAATCTCTT TTGGCCACAG CTATCTCTCC TTTNCTNAAT TAACTCATAT
101 AGTTTAACGT AATATGTCAT ACTATCCAAA TAAAAAGCCA AAGCCAATGT
151 NCTATTGACT TTNACTTTTC ANATCATGAC AACATTCTAA TTGTATTGTT
201 TAATTATTTT NTGTCGTCGT CTTTNACTTC TTTAAATTCA GCATCTTCTA
251 CAGTACTATC ATTGTTTTNA CCAGCATTAG CACCTTGTNT TGTTGTTGCT
301 GTTGAGCCGC TTGCTCATAT ACTTTTNCTG NTAATTCTTG ANTCACTTTT
351 TCAAGTTCTT CTTTTTTAGA TTTANTATCT TCTATATNCT TGACCTTTCT
401 AANGCAGTTT TAAGAGCGTC TTTTTTCCTC TTTCTGCAGT TTTNTTATAC
451 TTCCTTTCAC CGTNATTTTT CGGCTTATTT CAGTTAAANG TTTTTCCANC
501 TTGGGTNTAN CTATGGCTAG NAAAGNTTCG NTTCCT

SEQ ID NO. 58
1116.t7 LENGTH: 537 nt
1 AAGATAAAAT GGCATTACAA CGTTTNAAAG ATGCTGCTGA AAAANCTAAA
51 AAAGACTTAT CAGGTGTATC ACAAACTCAA ATCTCATTAC CATTTATCTC
101 AGCTGGTGAA AACGGTCCAT TACACTTAGA AGTAAACTTA ACTCGTNCTA
151 AATTTGAAGA ATTATCAGAT TCATTAATTA GAAGANCAAT GGAACCTACA
201 CGCCAAGCAA TGAAAGACGC TGGCTTAACA AACTCAGATA TCGATGAAGT
251 TATCTTAGTT GGTGGNTCAA CTCGTATTCC AGCAGTACAA GANGCTGTCA
301 AAAAAGAAAT CGGTAAAGAG CCTAACAAAG GAGTAAACCC GGNCGAAGTA
351 GGTGGCAATG GGNGCTGCAA TCCAAGGTGG CGTTATTCAC AGGTGACGTT
401 TAAAGACGTG TATTATTAGG NCGTAACACC ACTATCTTTA GGTATTGAAA
451 TTTTAGGTGG NCGTATGNAT TACGGTAATT GAACGTAACA CTACGGTTCC
501 TNCATTCTAA NTCTCAAAAT CTNTTCAACA GCAGTT

Mutant: NT89

Phenotype: temperature sensitivity

Sequence map: Mutant NT89 is complemented by pMP122, which contains a 0.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 54, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal a high level of similarity at the peptide level to the *trmD* gene, encoding (guanine-N1-) methyltransferase (EC 2.1.1.31), from various prokaryotes, including *S. marcescens* (Genbank Accession No. L23334; published in Jin, S. *et al. Gene* 1 (1994) 147-148), *H. influenzae*, *E. coli*, and *S. typhimurium*. The predicted size and relative orientation of the TrmD ORF is depicted by an arrow.

DNA sequence data: The following DNA sequence data represent the sequences at the left-most and right-most edges of clone pM122, using standard M13 forward and M13 reverse sequencing primers. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing; it can also be used to demonstrate similarity to the *trmD* gene of *S. marcescens*:

clone pMP122, a 925 bp genomic fragment

SEQ ID NO. 59

pMP122.con Length: 925 nt

```
  1 CTAGAGTCGA TCTAAAGAAT ATNTAANTCC TNATATKSCT GATGTTGTAA
 51 AAGAAGTGGA TGTTGAAAAT AAAAAAATTA TCATCACGCC AATGGAAGGA
101 TTGTTGGATT AATGAAAATT GATTATTTAA CTTTATTTCC TGAAATGTTT
151 GATGGTGTTT TAAATCATTC AATTATGAAA CGTGCCCANG AAAACAATAA
201 ATTACAAATC AATACGGTTA ATTTTAGAGA TTATGCAATT AACAAGCACA
251 ACCAAGTAGA TGATTATCCG TATGGTGGCG GWCAAGGTAT GGTGTTAAAG
301 CCTGACCCTG TTTTTAATGC GATGGAAGAC TTAGATGTCA CAGAMCAAAC
351 ACGCGTTATT TTAATGTGTC CACAAGGCGA GCCATTTTCA CATCAGAAAG
401 CTGTTGATTT AAGCAAGGCC GACCACATCG TTTTCATATG CGGACATTAT
451 GAAGGTTACG ATGAACGTAT CCGAACACAT CTTGTCACAG RTGAAATATC
501 AATGGGTGAC TATGTTTTAA CTGGTGGAGA ATTGCCAGCG ATGACCATGA
551 CTGATGCTAT TGTTAGACTG ATTCCAGGTG TTTTAGGTAA TGNACAGTCA
601 CATCAAGACG ATTCATTTTC AGATGGGTTA TTAGAGTTTC CGCAATATAC
651 ACGTCCGCGT GAATTTAAGG GTCTAACAGT TCCAGATGTT TTATTGTCTG
701 GAAATCATGC CAATATTGAT GCATGGAGAC ATGAGCAAAA GTTGAACCGC
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751  ACATATAATN AAAGACCTGA CTTAATTNNA AAATACCCAT TAANCCAATG
801  GCAGCATAAG GCAAATCATT CAGNAAANAT CATTAAAATC AGGTATTNGT
851  AAAAAGGTTN AGTGATTGTG NNNAACNNAN TNGNATGTGG CAAACATNCN
901  AANTACATCC TGGAAGGACC TCACG
```

Mutant: NT94

Phenotype: temperature sensitivity

Sequence map: Mutant NT94 is complemented by pMP170, which contains a 2.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 55. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to *yabM*, a hypothetical ORF of uncharacterized function from *B. subtilis*, noted as being similar to the *spoVB* gene from *B. subtilis*; further similarities are noted to hypothetical ORFs from *E. coli* and *H. influenzae*.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP170, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP170

SEQ ID NO. 60
pMP170 Length: 2531 nt

```
  1  TGGYTTRTTT CAACATAATA TAGACATTTY CAATGTTATT CTATTAATTC
 51  TCCACGAAAC TGTTATCTTA TCGTTTTCTG GTTCTAATAT GTGTTTTTTG
101  GGTGATTTAA TTACTTGTTC CGTTGAACAT TTACAAGGCC TTTTTTAAGT
151  TAACTGTTTG ACCTCATTAC GTGTACCGAC GCCCATATTT GCTAAAAATT
201  TATCTATTCT CATCGTAAAA ACCTAACTCT ACGTCTTAAT TTTTCAGGAA
251  TTTCACCTAA GAATTCGTCC GCAAGACGCG TTTTAATTGT GAWTGTACCG
301  TAAATTAGAA TACCTACTGT AACACCTAAA ATAATAATGA TTAAGTWACC
351  AAGTTTTAGT AGGTYCTAAR AATARATTTG CAAGGNAAAA TACTAATTCT
401  ACACCTAGCA TCATAATNNT GNATACAAGG ATATWTWTGC AAAATGGATC
451  CCAACTATAG CTGAATTTAA ACTTCGCATA TWTTTTAAGR ATWTAGRAAT
501  TACATCCMAT TGCAAATAAT TAATGCGATA CTAGTACGTA AAATTGCACC
551  AGGTGTATGG AATAACATAA TTAATGGATA GTTTAACGCT AACTTGATAA
601  CTACAGAAGC TAAAATAACA TAAACTGTTA ATTTCTGTTT ATCTATACCT
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651  TGTAANATNG ATGCCGTTAC ACTTAATAGT GAAATYAGTA TTGCTACAGG
701  CGCATAATAK AATAATAAGC GACTACCATC ATGGTTAGGG TCATGACCTA
751  WAACAATTGG ATCGTAACCA TAGATAAACT GTGAAATTAA TGGTTGTGCC
801  AAGGCCATAA TCYCCAATAC TAGCTGGGAA CAGTTATAAA CATTWAGTTA
851  CACCAATTAG ATGTTCCTAA TTTGATGATG CATTTCATGT AAGCGACCTT
901  CTGCAAATGT TTTTGTAATA TAAGGAATTA AACTCACTGC AAAACCAGCA
951  CTTAATGATG TCGGAATCAT TACAATTTTA TTAGTTGACA TATTTAGCAT
1001 ATTAAAGAAT ATATCTTGTA ACTGTGAAGG TATACCAACT AAAGATAAAG
1051 CACCGTTATG TGTAAATTGA TCTACTAAGT TAAATAATGG ATAATTCAAA
1101 CTTACAATAA CGAACGGTGA TACTATAAGC AATAATTTCT TTATACATCT
1151 TGCCATATGA CACATCTATA TCTGTGTAAT CAGATTCGAC CATACGATCA
1201 ATATTATGCT TACGCTTTCT CCAGTAATAC CAGAGTGTGR ATATRCCAAT
1251 AATCGCACCA ACTGCTGCTG CAAAAGTAGC AATACCATTG GCTAATAAAA
1301 TAGAGCCATC AAAGACATTT AGTACTAAAT AACTTCCGAT TAATATGAAA
1351 ATCACGCGTG CAATTTGCTC AGTTACTTCT GACACTGCTG TTGGCCCCAT
1401 AGATTTATAA CCTTGGAATA TCCCTCTCCA TGTCGCTAAT ACAGGAATAA
1451 AGATAACAAC CATACTAATG ATTCTTATAA TCCAAGTTAA TATCATCCGA
1501 CTGACCAACC GTTTTTATCA TGAATGTTTC TAGCTAATGT TAATTCAGAA
1551 ATATAAGGTG YTAAGAAATA CAGTACCAAG AAACCTAAAA CACCGGTAAT
1601 ACTCATTACA ATAAAAYTCG ATTTATAAAA WTTCTGACTT WACTTTAWAT
1651 GCCCCAATAG CATTATATTT CGCAACATAT TTCGAAGCTG CTAATGGTAC
1701 ACCTGCTGTC GCCAACTGCA ATTGCAATAT TATATGGTGC ATAAGCGTWT
1751 GTTGAACGGS GCCATATTTT CTTGTCCCNC CAATTAAATA GTTGAATGGA
1801 ATGATAAAAA GTACGCCCAA TACCTTGGTA ATTAATATAC TAATGGTAAT
1851 TAAAAAGGTT CCACGCACCA TTTCTTTACT TTCACTCATT ACGAATCTCC
1901 CTATCTCATG TTTATTAAAG TTTTGTAAAC TAAAAGCTGT TTCTCTGTAA
1951 AATCATTTTT CATTATTATG AATATATCAC AAAACTTTAT TTCATYGTCG
2001 TATATTTCAA TGGAATTATC CATAACAAAA TTATCAACAC ATTGTCATTG
2051 AATACTAGAT TTTGATTAGA ATATTACGAA ATTTCATATA AACATTATAC
2101 TACTATTTGA GATGAACATC GCATAACAGT AGAAAAATCA TTCTTATCAT
2151 ACACATACAT CTTCATTTTT TATGAAGTTC ACATTATAAA TATATTCAAC
2201 ATAATTGTCA TCTCATAACA CAAGAGATAT AGCAAAGTTT AAAAAAGTAC
2251 TATAAAATAG CAATTGAATG TCCAGTAACA AATTTGGAGG AAGCGTATAT
2301 GTATCAAACA ATTATTATCG GAGGCGGACC TAGCGGCTTA ATGGCGGCAG
2351 TAGCWGCAAG CGAACAAAGT AGCAGTGTGT TACTCATTGA AAAAAAGAAA
2401 GGTCTAGGTC GTAAACTCAA AATATCTGGT GGCGGTAGAT GTAACGTAAC
2451 TAATCGAYTA CCATATGCTG AAATTATTCA AGGAACATTC CCTGGAAATG
2501 GGAAATTTTY ATCATAGTTC CCTTTTCAAT T
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Mutant: NT96

Phenotype: temperature sensitivity

Sequence map: Mutant NT96 is complemented by pMP125, which contains a 2.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 56, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal strong

similarities at the peptide level to the *murC* gene product, encoding UDP-N-Acetyl muramoyl-L-alanine synthase (EC 6.3.2.8), from *B. subtilis* (Genbank Accession No. L31845).

DNA sequence data: The following DNA sequence data represent the sequences at the left-most and right-most edges of clone pM125, using standard M13 forward and M13 reverse sequencing primers. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP125

SEQ ID NO. 61

pMP125.forward Length: 889 nt

```
  1 TCGAGCTCGG TACCCGGGGA TCCTCTAGAG TCGATCTACA GAGCTGTTTA
 51 ACGTTTGTAC TGAGTCACCG ATACCTTTAA CAGCATCTAC AACTGAGTTT
101 AAACGATCTA CTTTACCTTG GATATCCTCA GTTAAACGGT TTACTTTATG
151 AAGTAAATCT GTTGTTTCAC GAGTAATACC TTGAACTTGA CCTTCTACAC
201 CGTCAAGTGT TTTTGCAACA TAATCTAAGT TTTTCTTAAC AGAATTTAAT
251 ACAGCTACGA TACCGATACA TAAAATTAAG AATGCAATCG CAGCGATAAT
301 TCCAGCAATT GGTAAAATCC AATCCATTAA AAACGCCTCC TAATTAACAT
351 GTAATAATGT CATTAATAAT AAATACCCAT ACTACTCTAT TATAAACATA
401 TTAAAACGCA TTTTTCATGC CTAATTTATC TAAATATGCA TTTTGTAATT
451 TTTGAATATC ACCTGCACCC ATAAATGAAA ATAACAGCAT TATCAAATTG
501 TTCTAATACA TTAATAGAAT CTTCATTAAT TAACGATGCA CCTTCAATTT
551 TATCAATTAA ATCTTGTWTC GTTAATGCGC CAGTATTTTC TCTAATTGAT
601 CCAAAAATTT CACAATAAGA AATACACGAT CTGCTTTACT TAAACTTTCT
651 GCAAATTCAT TTAAAAATGC CTGTGTTCTA GAGAAAGTGT GTGGTTTGAN
701 ATACTGCAAC AACTTCTTTA TGTGGATATT TCTTTCGTGC GGTTTCAATT
751 GNNGCACTAA NTTCTCTTGG ATGGTGTNCA TAATCAGCTA CATTAACTTG
801 ATTTGCGATT GTAGTNTCAT NGANNGACGT TTAACNCCAC CAACGTTTCT
851 AATGCTTCTT TAANATTGGG ACATCTAACT TCTCTAAA
```

SEQ ID NO. 62

pMP125.reverse Length: 902 nt

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  1 GCATGCCTGC AGGTCGATCC AAAAATGGTT GAATTAGCTC CTTATAATGG
 51 TTTGCCMMMT TTRGTTGCCA CCGKTAATTA CAGATGTCMA AGCCAGCTAC
101 ACAGAGTTTG AAAAKGGSCC STWGAAAGGA AATGGAACGA ACGTKATAAG
151 TTATTTGCCA CATTACCATG TACGTAATAT AACAGCCATT TAACAAAAAA
201 GCCACCATAT GATGAAAGAW TGCCAAAAAT TGTCATTGTA ATTGATGAGT
251 TGGCTGATTT AATGATGATG GCTCCGCAAG AAGTTGAACA GTCTATTGCT
301 AGAATTGCTC AAAAAGCGAG AGCATGTGGT ATTCATATGT TAGTAGCTAC
351 GCAAAGACCA TCTGTCAATG TAATTACAGG TTTAATTAAA GCCAACATAC
401 CAACAAGAAT TGCATTTATG GTATCATCAA GTGTAGATTC GAGAACGATA
451 TTAGACAGTG GTGGAGCAGA ACGCTTGTTA GGATATGGCG ATATGTTATA
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501  TCTTGGTAGC GGTATGAATA AACCGATTAG AGTTCAAGGT ACATTTGTTT
551  CTGATGACGA AATTGATGAT GTTGTTGATT TTATCAAACA ACAAAGAGAA
601  CCGGACTATC TATTTGAAGA AAAAAGAAAT TGTTGAAAAA AACACAAACA
651  CMATCMCMAG ATGAATTATT TGATGATGTT TGTGCATTTA TGGTTAATGA
701  AGGACATATT TCAACATCAT TAATCCAAAG ACATTTCCAA ATTGGCTATA
751  ATAGAGCAGC AAGAATTATC GATCAATTAG AAGCAACTCG GTTATGTTTC
801  GAGTGCTAAT NGGTTCAAAA ACCNAGGGAT GTTTATGTTA CGGAAGCCGA
851  TTTTAAATAA AGAATAATTT ATGATTAAGG ATTTTTATAT AATGGACACC
901  CC
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Mutant: NT99

Phenotype: temperature sensitivity

Sequence map: Mutant NT99 is complemented by pMP176, which contains a 3.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 57. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to the *murG* gene, encoding UDP-GlcNAc:undecaprenyl-pyrophosphoryl-pentapeptide transferase, from *B. subtilis* (Genbank Accession No. D10602; published in Miyao, A. *et al. Gene* 118 (1992) 147-148.) Cross complementation studies (data not shown) have demonstrated that the minimal amount of clone pMP176 required for restoring a wild-type phenotype to mutant NT99 is contained in the right-half of the clone and contains the entire (predicted) *murG* ORF; the predicted size and orientation of this ORF is depicted in the restriction map by an arrow.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP176, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP176

SEQ ID NO. 63

pMP176 Length: 3592 nt

1	GATCCTTATT	CTGAATATTT	AACAAAWGCA	ACAAACGAAA	TCCCTTTGAA
51	TGAAAGGTGT	TTCAGGTGCA	TTTTKTAGGT	ATTGGTGCAG	AAAATGCAAA
101	AGAAAAATGA	ATCAAATTAT	GGTTACTAGT	CCTATGAAGG	GWTCTCCAGC
151	AGAACGTGCT	GGCATTCGTC	CTAAAGATGT	CATTACTAAA	GTAAATGGAA
201	AATCAATTAA	AGGTAAAGCA	TTAGATGAAG	TTGTCAAAGA	TGTTCGTGGT
251	AAAGAAAACA	CTGAAGTCAC	TTTAACTGTT	CAACGAGGTA	GTGAAGAAAA
301	AGACGTTAAG	ATTAAACGTG	RAAAAATTCA	TGTTAAAAGT	GTTGAGTATW
351	AGRAAAAAGG	TAAAGTTGGA	GTTATTACTA	TTAATAAATT	CCAGAMTGAT
401	ACATCCAGGT	GRATTGAAAG	ATGCAGTTCT	AAAAGCTCAC	CAAAGATGGT
451	TTGWAAAAGA	TTGTTTTAGA	TTTAAGAAAT	AATCCAGGTG	GACTACTAGA
501	TGAAGCTGTT	AAAATGGCAA	ATATTTTTAT	CGATAAAGGA	AAAACTGTTG
551	TTAAACTARA	AAAAGGTAAA	GATACTGAAG	CAATTCNNAC	TTCTAATGAT
601	GCGTTAAAAG	AAGCGAAAGA	CATGGATATA	TCCATCTTAG	TGAATGAAGG
651	TTCNGCTNGC	GCTTCTGAAG	TGTTTACTGG	TGCGCTAAAA	GACTNTAATA
701	AAGCTAAAGT	TTATGGGTCA	AAAACATTCG	GCAAAGGTGT	CGTACAAACT
751	ACAAGAGAGT	TTAAGGGATG	GTTCATTGTT	AAAATATACT	GAAATGGAAA
801	TGGTTAACGC	CAGATGGTCA	TTATATTCAC	NGTACAAGGC	ATNAAACCAG
851	ACGTTACTNT	TTGACACACC	TGAAATANCA	ATCTTTTAAA	TGTCATTCCT
901	AATACGANAA	CATTTAAAGT	TNGGAGACGA	TGAATCTAAA	ATATTAAAAC
951	TATTAAAAWT	GGTTTATCAG	CTTTAGGTTA	TAAAGTTGAT	AAATGGAATC
1001	AACGCCAATT	TGGATAAAGC	TTTAGAAAAT	CAAGTTAAAG	CTTYCCAMCA
1051	AGCGAATAAA	CTTGAGGTAM	YKGGKGAWTT	TAATAAAGAA	ACGAATAATA
1101	AATTTACTGA	GTTATTAGTT	GAAAAAGCTA	ATAAACATGA	TGATGTTCTC
1151	GATAAGTTGA	TTAATATTTT	AAAATAAGCG	ATACACACTA	CTAAAATTGT
1201	ATTATTATTA	TGTTAATGAC	ACGCCTCCTA	AATTTGCAAA	GATAGCAATT
1251	TAGGAGGCGT	GTTTATTTTT	ATTGACGTCT	AACTCTAAAA	GATATAAATT
1301	AGACATTTAC	AAATGATGTA	AATAACGCAA	TTTCTATCAT	CGCTGATAAC
1351	AATTCATGGT	TTAATATGCA	ATGAGCATAT	ACTTTTTAAA	TAGTATTATT
1401	CACTAGTTTT	AACAATCAAT	TAATTGGTAT	ATGATACTTT	TATTGGTTAT
1451	TTTTATCCCA	TAGTGTGATA	AWTACTATTT	TTCATTCAYA	ATAAAGGTTT
1501	AAAGCATGTT	AATAGTGTGT	TAAGATTAAC	ATGTACTGAA	AAACATGTTT
1551	WACAATAATG	AATATAAGGA	KTGACGTTAC	ATGAWCCGTC	CTAGGTAAAA
1601	TGTCMGAWTT	AGATCAAATC	TTAAATCTAG	TAGAAGAAGC	AAAAGAATTA
1651	ATGAAAGAAC	ACGACAACGA	GCAATGGGAC	GATCAGTACC	CACTTTTAGA
1701	ACATTTTGAA	GAAGATATTG	CTAAAGATTA	TTTGTACGTA	TTAGAGGAAA
1751	ATGACAAAAT	TTATGGCTTT	ATTGTTGTCG	ACCAAGACCA	AGCAGAATGG
1801	TATGATGACA	TTGACTGGCC	AGTAAATAGA	GAAGGCGCCT	TTGTTATTCA
1851	TCGATTAACT	GGTTCGAAAG	AATATAAAGG	AGCTGCTACA	GAATTATTCA
1901	ATTATGTTAT	TGATGTAGTT	AAAGCACGTG	GTGCAGAAGT	TATTTTAACG
1951	GACACCTTTG	CGTTAAACAA	ACCTGCACAA	GGTTTATTTG	CCAAATTTGG
2001	ATTTCATAAG	GTCGGTGAAC	AATTAATGGA	ATATCCGCCM	TATGATAAAG
2051	GTGAACCATT	TTATGCATAT	TATAAAAATT	TAAAAGAATA	GAGGTAATAT
2101	TAATGACGAA	AATCGCATTT	ACCGGAGGGG	GAACAGTTGG	ACACGTATCA
2151	GTAAATTTWA	RTTTAATTCC	AACTGCATTA	TCACAAGGTT	ATGGARGCGC
2201	TTTATATTGG	TTCTAAAAAT	GGTATTGAAA	GAGAGAATGA	TTGAWTCACC
2251	AACTACCCRG	AAATTAAGTA	TTATCCTATT	TCGGAGTGKT	AAATTAAGAA
2301	GATATATTTC	TTTAGAAAAT	GCCAAAGACG	TATTTAAAGT	ATTGAAAGGT
2351	ATTCTTGATG	CTCGTAAAGT	TTTGAAAAAA	GAAAAACCTG	ATCTATTATT
2401	TTCAAAAGGT	GGATTTGTAT	CTGTGCCTGT	TGTTATTGCA	GCCAAATCAT
2451	TAAATATACC	AACTATTATT	CATGAATCTG	ACTTAACACC	AGGATTAGCG
2501	AATAAGATAG	CACTTAAATT	TGCCAAGAAA	ATATATACAA	CATTTGAAGA

2551 AACGCTAAAC TACTTACCTA AAGAGAAAGC TGATTTTATT GGAGCAACAA
2601 TTCGAGAAGA TTTAAAAAAT GGTAATGCAC ATAATGGTTA TCAATTAACA
2651 GGCTTTWATG RAAATAAAAA AGTTTTACTC GTYATGGGTG GAAGCTTWGG
2701 AAGTAAAAAA TTAAATAGCA TTATTCGCGA AAACTTAGAT GCATTTATTA
2751 CAACAATATC AAGTGATACA TTTAACTGGT AAAGGATTAA AAGATGCTCA
2801 AGTTAAAAAA TCAGGATATA TACAATATGA ATTTGTTAAA GNGGATTTAA
2851 CAGATTTATT AGCAATTACG GATACAGTAA TAAGTAGAGC TGGATCAAAT
2901 GCGATTTATG GAGTTCTTAA CATTACGTNT ACCAATGTTA TTAGTACCAT
2951 TAGGTTTAGA TCAATCCCGA GGCGACCAAA TTGACANTGC AAATCATTTT
3001 GCTGATAAAG GATATGCTAA AGCGATTGAT GAAGAACAAT TAACAGCACA
3051 AATTTTATTA CAAGAACTAA ATGAAATGGA ACAGGAAAGA ACTCGAATTA
3101 TCAATAATAT GAAATCGTAT GAACAAAGTT ATACGAAAGA AGCTTTATTT
3151 GATAAGATGA TTAAAGACGC ATTGAATTAA TGGGGGGTAA TGCTTTATGA
3201 GTCAATGGAA ACGTATCTCT TTGCTCATCG TTTTTACATT GGTTTTTGGA
3251 ATTATCGCGT TTTTCCACGA ATCAAGACTT GGGAAATGGA TTGATAATGA
3301 AGTTTATGAG TTTGTATATT CATCAGAGAG CTTTATTACG ACATCTATCA
3351 TGCTTGGGGC TACTAAAGTA GGTGAAGTCT GGGCAATGTT ATGTATTTCA
3401 TTACTTCTTG TGGCATATCT CATGTTAAAG CGCCACAAAA TTGAAGCATT
3451 ATTTTTTGCA TTAACAATGG CATTATCTGG AATTTTGAAT CCAGCATTAA
3501 AAAATATATT CGATAGAGAA AGGACCTGAC ATTGCTGGCG TTTGAATTGG
3551 ATGATTAACA GGRTTTAGTT TTCCTGAGCG GTCATGCTAT GG

Mutant: NT102

Phenotype: temperature sensitivity

Sequence map: Mutant NT102 is complemented by pMP129, which contains a 2.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 58 (there are no apparent restriction sites for EcoR I, Hind III, Bam HI or Pst I). Database searches at both the nucleic acid and peptide levels reveal strong similarity to one hypothetical ORF of unknown function from *Synechocystis spp.*; another ORF with no apparent homolog on the current databases is also predicted to be contained in this clone. The predicted sizes and orientations of these two hypothetical ORFs is depicted in the map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP129, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of

amplification from genomic DNA with subsequent DNA sequencing.

clone pMP129

SEQ ID NO. 64

pMP129 Length: 2573 nt

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   1 ATTCGAGCTC GGTACCCGKG GATCCTSYAG AGTCGATCCG CTTGAAACGC
  51 CAGGCACTGG TACTAGAGTT TTGGGTGGTC TTAGTTATAG AGAAAGCCAT
 101 TTTGCATTGG AATTACTGCA TCAATCACAT TTAATTTCCT CAATGGATTT
 151 AGTTGAAGTA AATCCATTGA TTGACAGTAA TAATCATACT GCTGAACAAG
 201 CGGTTTCATT AGTTGGAACA TTTTTTGGTG AAACTTTATT ATAAATAAAT
 251 GATTTGTAGT GTATAAAGTA TATTTTGCTT TTTGCACTAC TTTTTTTAAT
 301 TCACTAAAAT GATTAAGAGT AGTTATAATC TTTAAAATAA TTTTTTTCTA
 351 TTTAAATATA TGTTCGTATG ACAGTGATGT AAATGATTGG TATAATGGGT
 401 ATTATGGAAA AATATTACCC GGAGGAGATG TTATGGATTT TTCCAACTTT
 451 TTTCAAAACC TCAGTACGTT AAAAATTGTA ACGAGTATCC TTGATTTACT
 501 GATAGTTTGG TATGTACTTT ATCTTCTCAT CACGGTCTTT AAGGGAACTA
 551 AAGCGATACA ATTACTTAAA GGGATATTAG TAATTGTTAT TGGTCAGCAG
 601 ATAATTWTGA TATTGAACTT GACTGCMACA TCTAAATTAT YCRAWWYCGT
 651 TATTCMATGG GGGGTATTAG CTTTAANAGT AATATTCCAA CCAGAAATTA
 701 GACGTGCGTT AGAACAACTT GGTANAGGTA GCTTTTTAAA ACGCNATACT
 751 TCTAATACGT ATAGTAAAGA TGAAGAGAAA TTGATTCAAT CGGTTTCAAA
 801 GGCTGTGCAA TATATGGCTA AAAGACGTAT AGGTGCATTA ATTGTCTTTG
 851 AAAAAGAAAC AGGTCTTCAA GATTATATTG AAACAGGTAT TGCCAATGGA
 901 TTCAAATATT TCGCAAGAAC TTTTAATTAA TGTCTTTATA CCTAACACAC
 951 CTTTACATGA TGGTGCAAKG ATTATTCAAG GCACGAARAT TGCAGCAGCA
1001 GCAAGTTATT TGCCATTGTC TGRWAGTCCT AAGATATCTA AAAGTTGGGT
1051 ACAAGACATA GAGCTGCGGT TGGTATTTCA GAAGTTATCT GATGCATTTA
1101 CCGTTATTGT ATCTGAAGAA ACTGGTGATA TTTCGGTAAC ATTTGATGGA
1151 AAATTACGAC GAGACATTTC AAACCGAAAT TTTTGAAGAA TTGCTTGCTG
1201 AACATTGGTT TGGCACACGC TTTCAAAAGA AAGKKKTGAA ATAATATGCT
1251 AGAAAKTAAA TGGGGCTTGA GATTTATTGC CTTTCTTTTT GGCATTGTTT
1301 TTCTTTTTAT CTGTTAACAA TGTTTTTGGA AATATTCTTT AAACACTGGT
1351 AATTCTTGGT CAAAAGTCTA GTAAAACGGA TTCAAGATGT ACCCGTTGAA
1401 ATTCTTTATA ACAACTAAAG ATTTGCATTT AACAAAAGCG CCTGAAACAG
1451 TTAATGTGAC TATTTCAGGA CCACAATCAA AGATAATAAA AATTGAAAAT
1501 CCAGAAGATT TAAGAGTAGT GATTGATTTA TCAAATGCTA AAGCTGGAAA
1551 ATATCAAGAA GAAGTATCAA GTTAAAGGGT TAGCTGATGA CATTCATTAT
1601 TCTGTAAAAC CTAAATTAGC AAATATTACG CTTGAAAACA AAGTAACTAA
1651 AAAGATGACA GTTCAACCTG ATGTAAGTCA GAGTGATATT GATCCACTTT
1701 ATAAAATTAC AAAGCAAGAA GTTTCACCAC AAACAGTTAA AGTAACAGGT
1751 GGAGAAGAAC AATTGAATGA TATCGCTTAT TTAAAAGCCA CTTTTAAAAC
1801 TAATAAAAAG ATTAATGGTG ACACAAAAGA TGTCGCAGAA GTAACGGCTT
1851 TTGATAAAAA ACTGAATAAA TTAAATGTAT CGATTCAACC TAATGAAGTG
1901 AATTTACAAG TTAAAGTAGA GCCTTTTAGC AAAAAGGTTA AAGTAAATGT
1951 TAAACAGAAA GGTAGTTTRS CAGATGATAA AGAGTTAAGT TCGATTGATT
2001 TAGAAGATAA AGAAATTGAA TCTTCGGTAG TCGAGATGAC TTMCAAAATA
2051 TAAGCGAAGT TGATGCAGAA GTAGATTTAG ATGGTATTTC AGAATCAACT
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2101  GAAAAGACTG TAAAAATCAA TTTACCAGAA CATGTCACTA AAGCACAACC
2151  AAGTGAAACG AAGGCTTATA TAAATGTAAA ATAAATAGCT AAATTAAAGG
2201  AGAGTAAACA ATGGGAAAAT ATTTTGGTAC AGACGGAGTA AGAGGTGTCG
2251  CAAACCAAGA ACTAACACCT GAATTGGCAT TTAAATTAGG AAGATACGGT
2301  GGCTATGTTC TAGCACATAA TAAAGGTGAA AAACACCCAC GTGTACTTGT
2351  AGGTCGCGAT ACTAGAGTTT CAGGTGAAAT GTTAGAATCA GCATTAATAG
2401  CTGGTTTGAT TTCAATTGGT GCAGAAGTGA TGCGATTAGG TATTATTTCA
2451  ACACCAGGTG TTGCATATTT AACACGCGAT ATGGGTGCAG AGTTAGGTGT
2501  AATGATTTCA GCCTCTCATA ATCCAGTTGC AGATAATGGT ATTAAATTCT
2551  TTGSCTCGAC CNCCNNGCTN GCA
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Mutant: NT114

Phenotype: temperature sensitivity

Sequence map: Mutant NT114 is complemented by pMP151, which contains a 3.0 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 59. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to the *dfp* gene, encoding a flavoprotein affecting pantothenate metabolism and DNA synthesis, from *E.coli* (Genbank Accession No. L10328; published in Lundberg, L.G. *et al. EMBO J.* 2 (1983) 967-971). The predicted size and orientation of the Dfp ORF is represented by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP151, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP151

SEQ ID NO. 65

pMP151 Length: 2976 nt

```
  1  GRTCGACTCT AGAGTCGATC TTTAAATGGG TCTCTTTCAA CAACCGCGTC
 51  ATATTTTTMA ACATAACCTT TTTTRATAAG TCCATCTAAA CTGGATTTTR
101  AAAAGCCCAT ATCCTCAATA TCAGTTAAAA ATATTGTTTT ATGTTGTTCT
151  TCAGACAAGT AAGCATACAA ATCGTATTGT TTAATAACTT TCTCCAACTT
201  AGCTAATACT TCATCAGGAT GATACCCTTC AATGACACGA ACAGCACGCT
251  TGGTTTTTTT AGTTATATTT TGTGTGAGAA TCGTTTTTTC TTCAACGATA
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301	TCATCTTTTA	ACAACTTCAT	AAGCAATTGA	ATATCATTAT	TTTTTTGCGC
351	ATCTTTATAA	TAATAGTAAC	CATGCTTATC	AAATTTTTGT	AATAAAGCTG
401	AAGGTAGCTC	TATGTCATCT	TTCATCTTAA	ATGCTTTTTT	ATACTTCGCT
451	TTAATAGCAC	TCGGAAGCAT	CACTTCTAGC	ATAGAAATAC	GTTTAATGAC
501	ATGAGTTGAA	CCCATCCACT	CACTTAAAGC	TATTAATTCT	GATGTTAATT
551	CTGGTTGTAT	ATCTTTCACT	TCTATGATTT	TTTTTAACTT	CGAAACGTCA
601	AGTTGTGCAT	CAGGTTCTGC	TGTTACTTCC	ATTACATAAC	CTTGAATCGT
651	TCTTGGTCCA	AAAGGTACAA	TTACACGCAC	ACCAGGTTGG	ATGACAGATT
701	CGAGTTGTTC	GGGAATTATA	TAATCAAATT	TATAGTCAAC	GCTCTTCGAC
751	GCGACATCGA	CTATGACTTT	CGCTATCATT	ATKGCCACCT	AGTTTCTAGT
801	TCATCTAAAA	TTTGTGCAGC	WAATACTACK	TTTTKNCCTT	YCTTGATATT
851	TACKTTTTCA	TTAKTTTTAA	AATGCATTGT	CAATTCATTA	TCATCAGAAC
901	TAAATCCGAT	AGACATATCC	CCAACATTAT	TTGAAATAAT	CACATCTGCA
951	TTTTTCTTGC	GTAATTTTTG	TTGTGCATAA	TTTTCAATAT	CTTCAGTCTC
1001	TGCTGCAAAG	CCTATTAAAT	ACTGTGATGT	TTTATGTTCA	CCTAAATATT
1051	TAAGAATGTC	TTTAGTACGT	TTAAAAGATA	CTGACAAATC	ACCATCCTGC
1101	TTTTTCATCT	TATGTTCCTA	ATACATCAAC	CGGTGTATAG	TCAGATACGG
1151	CTGCTGCTTT	TACAACAATA	TYTTGTTCCG	TYAAATCGGC	TTGTCACTTG
1201	GTTCAAACAT	TTCTTCAGGC	ACTTTGRACA	TGAATAACTT	CAATATCTTT
1251	TGGATCCTCT	AGTGTTGTAG	GACCAGCAAC	TAACGTCACG	ATAGCTCCTC
1301	GATTTCGCAA	TGCTTCAGCT	ATTGCATAGC	CCATTTTTCC	AGAAGAACGA
1351	TTGGATACAA	ATCTGACTGG	ATCGATAACT	TCAATAGTTG	GTCCTGCTGT
1401	AACCAATGCG	CGTTTATCTT	GAAATGAACT	ATTAGCTAAA	CGATTACTAT
1451	TTTGAAAATG	AGCATCAATT	ACAGAAACGA	TTTGAAGCGG	TTCTTCCATA
1501	CGTCCTTTAG	CAACATAACC	ACATGCTAGA	AATCCGCTTC	CTGGTTCGAT
1551	AAAATGATAC	CCATCTTCTT	TTAAAATATT	AATATTTTGC	TGCGTTACGT
1601	TTATTTTCAT	ACATATGCAC	ATTCATAGCA	GGCGCAATAA	ATTTCGGTGT
1651	CTCTGTTGCT	AGCAACGTTG	ATGTCACCAA	ATCATCAGCA	ATACCTACAC
1701	TCAATTTTGC	AATTGTATTT	GCCGTTGCAG	GTGCAACAAT	GATTGCATCK
1751	GCCCAATCCA	CCTAATGCAA	TATGCTGTAT	TTCTGGAAGG	ATTTTYTTCT
1801	ATAAAAGTAT	CTGTATAAAC	AGCATTTCGA	MTTATTGCTT	GAAATGCTAA
1851	TGGTGTCACA	AATTTTTGTG	CGTGATTCGT	TAAACATAAC	GCGAACTTCA
1901	TAACCCAGAT	TGTGTTAACT	TACTTGTCAA	ATCAATTGCT	TTATATGCCG
1951	CAATGCCACC	TGTAACGGCT	AATAATATTT	TCTTCATATT	CAATCTCCCT
2001	TAAATATCAC	TATGACATTT	ACGCTTTACA	TCATCATATG	CGCACAAATG
2051	CTCATTACTT	TTTTATAGAT	ACAAATTTAG	TATTATTATA	ACATCAATCA
2101	TTGGATAAAC	TAAAAAAACA	CACCTACATA	GGTGCGTTTG	ATTTGGATAT
2151	GCCTTGACGT	ATTTGATGTA	ACGTCTAGCT	TCACATATTT	TTAATGGTCG
2201	AAACTATTCT	TTACCATAAT	AATCACTTGA	AATAACAGGG	CGAATTTTAC
2251	CGTCAGCAAT	TTCTTCTAAC	GCTCTACCAA	CTGGTTTAAA	TGAATGATAT
2301	TCACTTAATA	ATTCAGTTTC	AGGTTGTTCA	TCAATTTCAC	GCGCTCTTTT
2351	CGCTGCAGTT	GTTGCAATTA	AATACTTTGA	TTTAATTTGT	GACGTTAATT
2401	GGTTTAAAGG	TGGATTTAAC	ATTATTTTTT	AGCCTCCAAA	ATCATTTTTC
2451	TATACTTAGC	TTCTACGCGC	TCTCTTTTTA	AGTGCTCAGC	TTCTACAATA
2501	CATTGAATTC	TATTCTTCGC	AAGTTCTACT	TCATCATTAA	CTACAACGTA
2551	ATCGTATAAA	TTCATCATTT	CAACTTCTTT	ACGCGCTTCG	TTAATACGAC
2601	TTTGTATTTT	CTCATCAGAT	TCTGTTCCTC	TACCTACTAA	TCGCTCTCTC
2651	AAGTGTTCTA	AACTTGGAGG	TGCTAAGAAA	ATAAATAGCG	CATCTGGAAA
2701	TTTCTTTCTA	ACTTGCTTTG	CACCTTCTAC	TTCAATTTCT	AAAAATACAT
2751	CATGACCTTC	GTCCATTGTA	TCTTTAACAT	ATTGAACTGG	TGTACCATAA
2801	TAGTTGCCTA	CATATTCAGC	ATATTCTATA	AATTGGTCAT	CTTTGATTAA

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2851  AGCTTCAAAC GCATCCCTAG TTTTAAAAAA GTAATCTACG CCATTCAACW
2901  TCACCTTCAC GCATTTGACG TGTTGTCATT GGAATAGRAG AGCTTRANNG
2951  ATGTATNGNG ATCGACCTGC AGTCAT
```

Mutant: NT124

phenotype: temperature sensitivity

Sequence map: Mutant NT124 is complemented by plasmid pMP677, which carries a 3.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 60 with open boxes to depict the current status of the contig project; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal no significant similarities to known genes at this time.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP677, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed later via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP677

SEQ ID NO. 66

pMP677.forward Length: 540 nt

```
  1  TACCCGGGGA CCTTGAAAAA TACCTGGTGT ATCATACATA AATGANGTGT
 51  CATCTANAGG AATATCTATC ATATCTNAAG TTGTTCCAGG GANTCTTGAA
101  GTTGTTACTA CATCTTTTTC ACCAACACTA GCTTCAATCA GTTTATTAAT
151  CAATGTAGAT TTCCCAACAT TCGTTGTCCC TACAATATAC ACATCTTCAT
201  TTTCTCGAAT ATTCGCAATT GATGATAATA AGTCNTNTNT GCCCCAGCCT
251  TTTTCAGCTG AAATTAATAC GACATCGTCA GCTTCCAAAC CATATTTTCT
301  TGCTGTTCGT TTTAACCATT CTTTAACTCG ACGTTTATTA ATTTGTTTCG
351  GCAATAAATC CAATTTATTT GCTGCTAAAA TGATTTTTTT GTTTCCGACA
401  ATACGTTTAA CTGCATTAAT AAATGATCCT TCAAAGTCAA ATACATCCAC
451  GACATTGACG ACAATACCCT TTTTATCCGC AAGTCCTGAT AATAATTTTA
501  AAAAGTCTTC ACTTTCTAAT CCTACATCTT GAACTTCGTT
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SEQ ID NO. 67

pMP677.reverse Length: 519 nt

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  1 GACGCGTAAT TGCTTCATTG AAAAAATATA TTTGTNGAAA GTGGTGCATG
 51 ACAAATGTAC TGCTCTTTTT GTAGTGTATC AGTATTGTGA TGTTTTAATG
101 AGAATATTAT ATGAATCATT ATGAAATTTA ATAAAAATAA AAGAAATGAT
151 TATCATTTTT TCTTATATAC TGTTAAACGG TTTGGAATTT TTAGGTATAC
201 ACTGTATTGG TTGATATAAC TCAACTAATA ATTGCGAACA GAGTATTTCA
251 AATTGAAAAG TATTATGAGC GTGATACATA ATCAAAATTG TAGGCTCAAG
301 AACCACTACA TAATAAACCA TAAGCGGTTC TTTATCATTT ATGTCTCGCT
351 CTCAAATGTA AATTAATAAT TGTTTTGGGG GAGTTTGAAG TTAAATATTT
401 AACAGGATTT ATTTTAATAT TATTGTTAGA AGGAATTTTT ACAAATTCAG
451 CGAGTGCAAT CGAATATTCA GACTTACATC ATAAAAGTAA GTTTGATTCA
501 AAGCGTCCTA AGTTAATGC
```

Mutant: NT125

Phenotype: temperature sensitivity

Sequence map: Mutant NT125 is complemented by plasmid pMP407, which carries a 3.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 61. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide level similarities to *rnpA* (Genbank Accession No. X62539), encoding the protein component of RNAseP (EC 3.1.26.5), and *thdF* (Genbank Accession No. X62539), a hypothetical ORF with similarities to the thiophene/furan oxidase from *E. coli*.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP407, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP407

SEQ ID NO. 68

pMP407 Length: 3308 nt

```
  1 ACCAATATAT GCATCTGAAC GACTTAATAT CTTTTCGCCT GTGTTTAACA
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51   CTTTACCTGC AGCGTTAATA CCTGCCATCA ATCCTTGTCC TGCTGCTTCT
101  TCATAACCAG ATGTACCATT AATTTGACCT GCAGTATATA AGTTTTTAAT
151  CATTTTCGTT TCAAGTGTAG GCCATAACTG CGTTGGCACA ATCGCATCAT
201  ATTCAATTGC GTAGCCGGCA CGCATCATAT CTGCTTTTTC AAGACCTGGT
251  ATCGTCTCTA ACATTTGACG TTGCACATGT TCAGGAAGAC TTGTNGACAA
301  TCCTTGCACA TATACTTCAT TTGTATTAAC GACCTTCAGG CTCTAAGAAA
351  AAGTTGATGT CGCGGCTTAT CATTAAATCG AACAAATTTA TCTTCAATTG
401  AAGGGCAATA ACGTGGCCCG GTTCCTTTAA TCATCCCTGA ATACATTGCA
451  GATAGATGTA AATTATCATC GATAACTTTG TGTGTTTCAN CATTAGTATA
501  CGTTAGCCAA CATGGCAATT GATCKAMYAT ATATTCTGTT GTTTCAAAGC
551  TGAATGCACG ACCTACATCG TCACCTGGTT GTATTTCAGT CTTCGAATAR
601  TCAATTGTTT TTGAATTGTA CACGGCGGWG GTGTACCTGT TTTAAAACGA
651  ACAATATCAA AACCAAGTTC TCTTARATGK GKSTGATAAT GTGATTGATG
701  GTAATTGGTG GATTTGGTCC ACTTGAATAC TTCATATTAC CTAAAATGAT
751  TTCACCACGT ATRAAATGTT GCCCGTWGTA ATAATTACTG CTTTAGATAA
801  ATACTCTGTA CCAATATTTG TACGTACACC TTKAACTGTC ATTAWCTTCT
851  ATAAKAAGTT CGTCTACCAT ACCTTGCATT AATATGCAAA TTTTCTTCAT
901  CTTCAATCAM GCGTTTCATT TCTTGTTGAT AAAGTACTWT AKCTGCTTGC
951  GCCKCTWAGT GCTCTTACAR CAGGTCCTTT AACTGTATTT AACATTCTCA
1001 TTTGAATGTG TGTTTTATCG ATTGTTTTTG CCATTTGTCC ACCTAAAGCA
1051 TCAATTTCAC GAACAACGAT ACCTTTAGCT GGTCCACCTA CAGATGGGTT
1101 ACATGGCATA AATGCAATAT TATCTAAATT TATTGTTAGC ATTAATGTTT
1151 TAGCACCACG TCTTGCAGAT GCTAAACCTG CTTCTACACC TGCATGTCCC
1201 GCACCTATAA CGATTACATC ATATTCTTGA ACCACAATAT AAACCTCCTT
1251 ATTTGATATC TTACTAGCCK TCTTAAGACG GTATTCCGTC TATTTCAATT
1301 ACTATTTACC TAAGCAGAAT TGACTGAATA ACTGATCGAT GAGTTCATCA
1351 CTTGCAGTCT CACCAATAAT TTCTCCTAAT ATTTCCCAAG TTCTAGTTAA
1401 ATCAATTTGT ACCATATCCA TAGGCACACC AGATTCTGCT GCATCAATCG
1451 CMTCTWGTAT CGTTTGTCTT GCTTGTTTTA ATAATGAAAT ATGTCTTGAA
1501 TTAGAAACAT AAGTCATATC TTGATTTTTG TACTTCTCCA CCAAAGAACA
1551 AATCTCGAAT TTGTATTTCT AATTCATCAA TACCTCCTTG TTTTAACATT
1601 GAAGTTTGAA TTAATGGCGT ATCACCTATC ATATCTTTAA CTTCATTAAT
1651 ATCTATGTTT TGCTCTAAAT CCATTTTATT AACAATTACG ATTACATCTT
1701 CATTTTTAAC CACTTCATAT AATGTGTAAT CTTCTTGAGT CAATGCTTCG
1751 TTATTGTTTA ATACAAATAA AATTAAGTCT GCTTGGCTAA GAGCCTTTCT
1801 AGAGCGTTCA ACACCAATCT TCTCTACTAT ATCTTCTGTC TCACGTATAC
1851 CAGCAGTATC AACTAATCTT AATGGCACGC CACGAACATT GACGTAMTCT
1901 TCTAAGACAT CTCTAGTAGT ACCTGCTACY TCAGTTACAA TCGCTTTATT
1951 ATCTTGTATT AAATTATTTA ACATCGATGA TTTACCTACG TTTGGTTTAC
2001 CAACAATAAC TGTAGATAAA CCTTCACGCC ATAATTTTAC CCTGCGCACC
2051 GGTATCTAAT AAACGATTAA TTTCCTGTTT GATTTCTTTA GACTGCTCTA
2101 AAAGAAATTC AGTAGTCGCA TCTTCAACAT CATCGTATTC AGGATAATCA
2151 ATATTCACTT CCACTTGAGC GAGTATCTCT AATATAGATT GACGTTGTTT
2201 TTTGATTAAG TCACTTAGAC GACCTTCAAT TTGATTCATC GCAACTTTAG
2251 AAGCTCTATC TGTCTTCGAG CGAWWAAAGT CCATAACTGY TTCAGCTTGA
2301 GATAAATCAA TACGACCATT TAAAAAGGCA MGTTTTGTAA ATTCAACCTG
2351 GCTCAGCCAT TCTAGCGCCA TATGTCATAG TAAGTTCCAG CACTCTATTA
2401 ATCGTTAAAA TACCACCATG ACAATTAATT TCTATAATAT CTTCGCGTGT
2451 AAATGTTTTT GGCGCTCTTA ACACAGACAC CATAACTTNT TCAACCATTC
2501 TTTAGACTCT GGATCAATAA TATGACCGTA ATTAATCGTA TGTGATGGAA
2551 CATCATTTAA AAGATGTTTT CCTTTATATA ATTTGTCAGC AATTTCAACG
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2601  GCTTGCGGTC CAGACAATCG AACAATTCCA ATTGCCCCTT CACCCATTGG
2651  TGTTGAAATA CTCGTAATTG TATCTAAATC CATATTGCTA CTCGCCTCCT
2701  TCAACGATGT GAATACATTT TAAAGTAAGT TATTATAACC CTAAGGTCAG
2751  TCTTAACGTT TGTCTGAGGT AAGACTTCGG GATGTGTTGA GTGGTTAATG
2801  TTTTCCTTCC CCTACCCTAT CCTTACTTAA TCTTTTTATT AAAAACTTTG
2851  GCAATTTTAA GTACGTGCTC AAGACTATTC TGTATTTGTA AAGTCGTCAT
2901  ATCTTTAGCT GGCTGTCTTG CTATTACAAT AATATCTTTG GCCAATATAT
2951  GCGACTTATG TACTTTGAAA TTTTCACGTA TTGCTCTTTT AATCTTGTTT
3001  CTTAACACTG CATTACCTAG TTTTTTAGAA ACACTAATAC CTAAGCGAAA
3051  ATGGTCTATT TCTTTATTAT TACAAGTGTA TACAACAAAT TGTCTGTTGG
3101  CTACAGAATG ACCTTTTTTA TATATTCTCT GAAAATCTGC ATTCTTTTTA
3151  ATTCGGTAAG CTTTTTCCAA TAACATCACT CGCTTATTTA TCGTTTTTAT
3201  TTGAAGCTAT ATTTAAACTT CTATTGAGCT TATAACATAA ATTTCTATTT
3251  ATTCTTAATT TAAACGAAAA AAAAGATCGA CTCTAGAGGA TCCCCGGGTA
3301  CCGAGCTC
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Mutant: NT144

Phenotype: temperature sensitivity

Sequence map: Mutant NT144 is complemented by plasmid pMP414, which carries a 4.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 62. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal identity to the Hsp70 locus from *S. aureus* (Genbank Accession No. D30690), including an additional 600 bp of unpublished sequence upstream of the Genbank entry. Experiments are underway to determine which ORF in this contig is the essential gene.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP414, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed later via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP414

SEQ ID NO. 69
pMP414.forward Length: 1004 nt

1 AGTTACGGCT TAATACTTGA ACCNAAAACC CAATTTTATA ATATGTATAG
51 AAAAGGCTTG CTCAAACTTG CTAATGAGGA TTTAGGTGCT GACATGTATC
101 AGTTGCTGAT GTCTAANATA GAACAATCTC CTTTCCATCA ATACGAAATA
151 TCTAATTTTG CATTAGATGG CCATGANTCN NAACATAATA AGGTTTACTG
201 GTTTAATGAG GAATATTATG GATTTGGAGC AGGTGCAAGT GGTTATGTAN
251 ATGGTGTGCG TTATACGAAT ATCAATCCAG TGAATCATTA TATCAAAGCT
301 ATNAATAAAG AAAGTAAAGC AATTTTAGTA TCAAATAAAC CTTCTTTGAC
351 TGAGAGAATG GAAGAAGAAA TGTTTCTTGG GTTGCGTTTA AATGAAAGTG
401 TGAGTAGTAG TAGGTTCAAA AAGAAGTTTG ACCAATCTAT TGAAAGTGTC
451 TTTGGTCAAA CAATAAATAA TTTAAAAGAG AAGGAATTAA TTGTAGAAAA
501 AGAACGATGT GATTGCACTT ACAAATAGAG GGAAAGTCAT ANGTAATGAG
551 GTTTTTGAAG CTTTCCTAAT CAATGATTAA GAAAAATTGA AATTTCGAGT
601 CTTTAACATT GACTTANTTT GACCAATTTG ATAAATTATA ATTAGCACTT
651 GAGATAAGTG AGTGCTAATG AGGTGAAAAC ATGANTACAG ATAGGCAATT
701 GAGTATATTA AACGCAATTG TTGAGGATTA TGTTGATTTT GGACAACCCG
751 TTGGTTCTAA AACACTAATT GAGCGACATA ACTTGAATGT TAGTCCTGCT
801 ACAATTAGAA ATGAGATGAA ACAGCTTGAA GATTTAAACT ATATCGAGAA
851 GACACATAGT TCTTCAGGGC GTTCGCCATC ACAATTAGGT TTTAGGTATT
901 ATGTCAATCG TTTACTTGAA CAAACATCTC ATCAAAAAAC AAATAAATTA
951 AGACGATTAA ATCAATTGTT AGTTGAGAAC AATATGATGT TTCATCAGCA
1001 TTGA

SEQ ID NO. 70

pMP414.reverse Length: 1021 nt

1 CCTGCAGGTC GATCCTGACA ACATTCTAAT TGTATTGTTT AATTATTTTT
51 TGTCGTCGTC TTTTACTTCT TTAAATTCAG CATCTTCTAC AGTACTATCA
101 TTGTTTTGAC CAGCATTAGC ACCTTGTGCT TGTTGTTGCT GTTGAGCCGC
151 TTGCTCATAT ACTTTTGCTG ATAATTCTTG AATCACTTTT TCAAGTTCTT
201 CTTTTTTAGA TTTAATATCT TCTATATCTT GACCTTCTAA AGCAGTTTTA
251 AGAGCGTCTT TTTTCTCTTC AGCAGATTTT TTATCTTCTT CACCGATATT
301 TTCGCCTAAA TCAGTTAAAG TTTTTTCAAC TTGGAATACT AGACTGTCAG
351 CTTCGTTTCT TAAGTCTACT TCTTCACGAC GTTTTTTATC TGCTTCAGCG
401 TTAACTTCAG CATCTTTTAC CATACGGTCR ATTTCTTCGT CTGATAATGA
451 AGAACTTGAT TGAATTGTAA TTCTTTGTTC TTTATTTGTA CCTAAGTCTT
501 TTGGCAGTTA CATTTACAAT ACCGTTTTTA TCGATATCAA ACGTTACTTC
551 AATTTGGAGG TTTACCACCG TTTCARMWGG TGGAATATCA GTCAATTGGA
601 ATCTACCAAG TGTTTTATTA TCCGCAGCCA TTGGACGTTC ACCTTGTAAT
651 ACGTGTACAT CTACTGATGG TTGATTATCT ACTGCTGTTG AATAGATTTG
701 AGATTTAGAT GTAGGAATCG TAGTGTTACG TTCAATTAAC GTATTCATAC
751 GTCCACCTAA AATTTCAATA CCTAAAGATA GTGGTGTTAC GTCTAATAAT
801 ACTACGTCTT TAACGTCACC TGTGATAACG CCACCTTGGA TTGCAGCTCC
851 CATTGCCACT ACTTCGTCCG GGTTTACTCC TTTGTTAGGC TCTTTACCGA
901 TTTCTTTTTT GACAGCTTCT TGTACTGCTG GAATACGAAT TGATCCACCA
951 ACTAAGATAA CTTCATCGAT ATCTGANTTT GTTAAGCCAG CGTCTTTCAT
1001 TGCTTGGCGT GTAGGTCCAT C

Mutant: NT152

Phenotype: temperature sensitivity

Sequence map: Mutant NT152 is complemented by plasmid pMP418, which carries a 3.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 63. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal limited peptide-level similarity to *yacF*, a hypothetical ORF, from *B. subtilis* (Genbank Accession No. D26185).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP418, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP418

SEQ ID NO. 71
pMP418 Length: 3010 nt

```
   1  ATGCCTGCAG GTCGATCACG ATGNAAGTCA TTCAATAAGA ATGATTATGA
  51  AAATAGAAAC AGCAGTAAGA TATTTTCTAA TTGAAAATCA TCTCACTGCT
 101  GTTTTTTAAA GGTTTATACC TCATCCTCTA AATTATTTAA AAATAATTAA
 151  TGGTATTTGA GCACGTTTAG CGACTTTATG ACTGACATTA CCAATTTCCA
 201  TTTCTTGCCA GATATTCAAA CCACGTGTAC TCAAAATGAT AGCTTGGTAT
 251  GTACCTCCAA TAGTAATTTC AATAACTTTG TCTGTTGAAC ACTAAGAGCA
 301  ATTTTAATTT CATAATGTGT TGTAAACATT TTTTTTGATT GGAGTTTTTT
 351  TCTGAGTTAA ACGATATCCT GATGTATTTT TAATTTTGCA CCATTTCCAA
 401  AAGGATAAGT GACATAAGTA AAAAGGCATC ATCGGGAGTT ATCCTATCAG
 451  GAAAACCAAG ATAATACCTA AGTAGAAAAG TGTTCAATCC GTGTTAAATT
 501  GGGAAATATC ATCCATAAAC TTTATTACTC ATACTATAAT TCAATTTTAA
 551  CGTCTTCGTC CATTTGGGCT TCAAATTCAT CGAGTARTGC TCGTGCTTCT
 601  GCAATTGATT GTGTGTTCAT CAATTGATGT CGAAGTTCGC TAGCGCCTCT
 651  TATGCCACGC ACATAGATTT TAAAGAATCT ACGCAAGCTC TTGAATTGTC
 701  GTATTTCATC TTTTTCATAT TTGTTAAACA ATGATAAATG CAATCTCAAT
 751  AGATCTAATA GTTCCTTGCT TGTGTGTTCG CGTGGTTCTT TTTCAAAAGC
 801  GAATGGATTG TGGAAAATGC CTCTACCAAT CATGACGCCA TCAATGCCAT
 851  ATTTTTCTGC CAGTTCAAGT CCTGTTTTTC TATCGGGAAT ATCACCGTTA
 901  ATTGTTAACA ATGTATTTGG TGCAATTTCG TCACGTAAAT TTTTAATAGC
 951  TTCGATTAAT TCCCAATGTG CATCTACTTT ACTCATTTCT TTACGTTGTA
1001  CGAAGATGAA TAGATAAATT GGCAATGTCT TGTTCGAAGA CAKTGCTTCA
1051  ACCAATCTTT CCATTCATCG ATTTCATAKT AGCCAAGGCG TGTTTTTAAC
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1101  ACTTTACCGG AASCCCACCT GCTTTAGTCG CTTGAATAAT TTCGGCAGCA
1151  ACGTCAGGTC TTAAGATTAA GCCGGANCCC TTACCCTTTT TAGCAACATT
1201  TGCTACAGGA CATCCCATAT TTAAGTCTAT GCCTTTAAAG CCCATTTTAG
1251  CTAATTGAAT ACTCGTTTCA CGGAACTGTT CTGGCTTATC TCCCCATATA
1301  TGAGCGACCA TCGGCTGTTC ATCTTCACTA AAAGTTAAGC GTCCGCGCAC
1351  ACTATGTATG CCTTCAGGGT GGCAAAAGCT TTCAGTATTT GTAAATTCAG
1401  TGAAAAACAC ATCCRGTCTA GNTGCTTCAN TTACAACGTG TCGAAAGACG
1451  ATATCTGTAA CGTCTTCCAT TGGCGCCAAA ATAAAAAATG GACGTGGTAA
1501  TTCACTCCAA AAATTTTCTT TCATAATATA TTTATACCCT CTTTATAATT
1551  AGTATCTCGA TTTTTTATGC ATGATGATAT TACCACAAAA GCNTAACTTA
1601  TACAAAAGGA ATTTCAATAG ATGCAACCAT TKGAAAAGGG AAGTCTAAGA
1651  GTAGTCTAAA ATAAATGTTG TGGTAAGTTG ATCAATACAA AGATCAAGGA
1701  TTATAGTATT AAATTGTTCA TTATTAATGA TACACTACTT ATGAATATGA
1751  TTCAGAATTT TCTTTGGCTA CTNCTTACAG TAAAGCGACC TTTTAGTTAT
1801  CTTATAACAA AGACAAATTT CTAAAGGTGA TATTATGGAA GGTTTAAAGC
1851  ATTCTTTAAA AAGTTTAGGT TGGTGGGATT NATTTTTTGC GATACCTATT
1901  TTTCTGCTAT TCGCATACCT TCCAAACTNT AATTTTATAA NCATATTTCT
1951  TAACATTGTT ATCATTATTT TCTTTTCCNT AGGTTTGATT TTAACTACGC
2001  ATATAATTAT AGATAAAAYT AAGAGCAACA CGAAATGAAT CATTAATACG
2051  GAATGTGATT AAAACATAAA ACTGAAGGAG CGATTACAAT GGCGACTAAG
2101  AAAGATGTAC ATGATTTATT TTTAAATCAT GTGAATTCAA ACGCGGTTAA
2151  GACAAGAAAG ATGATGGGAG AATATATTAT TTATTATGAT GGCGTGGTTA
2201  TAGGTGGTTT GTATGATAAT AGATTATTGG TCAAGGCGAC TAAAAGTGCC
2251  CAGCAGAAAT TGCAAGATAA TACATTAGTT TCGCCATATC CAGGTTTCTA
2301  AAGAAATGAT ATTAATTTTA GACTTTACCG AAGCAACAAA TCTCACTGAT
2351  TTATTTAAGA CCATAAAAAA TGATTTGAAA AAGTGAAGTA GTGAAGTGTG
2401  GGTGCAGAGA GAACTAAGCC CATCGWTAAA TGGTCGCTTG TTAAAGAAGA
2451  GTGACGGTCA CTCTTCTTTA TGTGCATATT TTATTTTGTC TGTTTBGTTA
2501  ACAAGCAGCA GTGTAACAAA TATGAGTAAG GATAAAATGA GTATAATATA
2551  GAAACCGAAT TTATCATTAA TTTCATTAAT CCATCTTCCT AAAAATGGAG
2601  CAATTAAACT TTGCAGTAAC AATGAAATTG ACGTCCATAT CGTAAATGAG
2651  CGACCGACAT ATTTATCTGA AACAGTGTTC ATTATAGCWG TATTCATATA
2701  AATTCTGATT GATGAAATTG AGTAGCCTAG TATAAAKGAT CCTATGAATA
2751  AGTAAAATGC TGAGTTTATC CAAATAAATA GTGCKGAATT TATGACTRRC
2801  TATGAAATAT AACAAAAATA TCACATACTT TAGKTGAGAT TTTCTTSGAA
2851  AGAATAGCTG AAATTAAACC TGCACATAAT CCTCCAATGC CATATAACAT
2901  ATCTGAAMAA CCAAAKTGTA CAGACCGAAA GTTTTAAAAC ATTATAAACA
2951  TATCCTGGTA ATGATATGTT AAAGATCGAC TCTAGAGGAT CCCCGGNTAC
3001  CGAGCTCGAA
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Mutant: NT156

phenotype: temperature sensitivity

Sequence map: Mutant NT156 is complemented by plasmids pMP672 and pMP679, which carry 4.5 kb inserts of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 64. Database searches at the nucleic acid

and (putative) polypeptide levels against currently available databases reveal identity to the *grlBA* locus, a known essential gene encoding DNA topoisomerase (EC 5.99.1.3), from *S. aureus* (Genbank Accession No. L25288; published in Ferrero, L. *et al.* *Mol. Microbiol.* **13** (1994) 641-653).

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP679, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed later via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clones pMP679 and pMP672

SEQ ID NO. 72

pMP679.forward Length: 548 nt

```
  1  ATCGGTACCC GGGGACCAAT ANACAGAAAG TATATTAAGT TTNGTAAATA
 51  ATGTACGTAC TNAAGATGGT GGTACACATG AAGTTGGTTT TAAAACAGCA
101  ATGACACGTG TATTTAATGA TTATGCACGT CGTATTAATG AACTTAAAAC
151  AAAAGATAAA AACTTAGATG GTAATGATAT TCGTGAAGGT TTAACAGCTG
201  TTGTGTCTGT TCGTATTCCA GAAGAATTAT TGCAATTTGA ANGACAAACG
251  AAATCTAAAT TGGGTACTTC TGAAGCTAGA AGTGCTGTTG ATTCAGTTGT
301  TGCAGACAAA TTGCCATTCT ATTTAGAAGA AAAAGGACAA TTGTCTAAAT
351  CACTTGTGGA AAAAAGCGAT TAAAGCACAA CAAGCAAGGG AAGCTGCACG
401  TAAAGCTCGT GAAGATGCTC GTTCAGGTAA GAAAAACAAG CGTAAAGACA
451  CTTTGCTATC TGGTAAATTA ACACCTGCAC AAAGTTAAAA ACACTGGAAA
501  AAAATGAATT GTATTTAGTC GAAGGTGATT CTGCGGGAAG TTCAGCAA
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SEQ ID NO. 73

pMP679.reverse Length: 541 nt

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  1  ACTGCAGGTC GAGTCCAGAG GWCTAAATTA AATAGCAATA TTACTAAAAC
 51  CATACCAATG TAAATGATAG CCATAATCGG TACAATTAAC GAAGATGACG
101  TAGCAATACT ACGTACACCA CCAAATATAA TAATAGCTGT TACGATTGCT
151  AAAATAATAC CTGTGATTAC TGGACTAATA TTATATTGCG TATTTAACGA
201  CTCCGCAATT GTATTAGATT GCACTGTGTT AAATACAAAT GCAAATGTAA
251  TTGTAATTAA AATCGCAAAT ACGATACCTA GCCATTTTTG ATTTAAACCT
301  TTAGTAATAT AGTAAGCTGG ACCACCACGG GAATCCACCA TCTTTATCAT
351  GTACTTTATA AACCTGAGCC AAAGTCGCTT CTATAAATGC ACTCGCTGCA
401  CCTATAAATG CAATAACCCA CATCCAAAAT ACTGCACCTG GACCGCCTAA
451  AACAATCGCA GTCGCAACAC CAGCAATATT ACCAGTACCA ACTCTCGAAC
501  CAGCACTAAT CGCAAATGCT TGGAATGGCG AAATACCCTT C
```

SEQ ID NO. 74
pMP672.forward Length: 558 nt

```
  1 AGGGTCTNNC ACGGTACCCG GGGNCCAATT WGATGAGGAG GAAATCTAGT
 51 GAGTGAAATA ATKCAAGATT TATCACTTGA AGATGTTTTA GGTGATCGCT
101 TTGGAAGATA TAGTAAATAT ATTATTCAAG AGCGTGCATT GCCAGATGTT
151 CGTGATGGTT TAAAACCAGT ACAACGTCGT ATTTTATATG CAATGTATTC
201 AAGTGGTAAT ACACACGATA AAAATTTCCG TAAAAGTGCG AAAACAGTCG
251 GTGATGTTAT TGGTCAATAT CATCCACATG GGAGACTCCT CAGTGTACGA
301 AGCAATGGTC CGTTTAAGTC AAGACTGGAA GTTACGACAT GTCTTAATAG
351 AAATGCATGG TAATAATGGT AGTATCGATA ATGATCCGCC AGCGGCAATG
401 CGTTACACTG AAGCTAAGTT AAGCTTACTA GCTGAAGAGT TATTACGTGA
451 TATTAATAAA GAGACAGTTT CTTTCATTCC AAACTATGAT GATACGACAC
501 TCCGAACCAA TGGTATTGCC ATCAAGAATT TCCTAACTTA CTAAKTGAAT
551 GGTTCTAC
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Mutant: NT160

Phenotype: temperature sensitivity

Sequence map: Mutant NT160 is complemented by plasmid pMP423, which carries a 2.2 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 65. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal identity to the Dlt locus of *S. aureus* (Genbank Accession No. D86240; unpublished). The pMP423 clone completely contains the genes *dltC*, encoding a putative D-Alanine carrier protein, and *dltD*, encoding a putative "extramembranal protein". Further subcloning and recomplementation experiments already in progress will demonstrate whether one or both of the ORFs encode essential genes.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP423, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP423

SEQ ID NO. 75

pMP423 Length: 2234 nt

```
   1 AGTCGATCTT TATTCTACAT GTCTCGTAAA AAATTATTGA AGAGTCAATT
  51 TGCAATGTCT AACGTGGCAT TCTTAATCAA CTTCTTCATA ATGGGAATTT
 101 GGCATGGTAT CGAAGTGTAT TACATTGTTT ATGGTTTATA CCATGCAGCA
 151 TTGTTTATAG GTTATGGCTA TTATGAACGT TGGCGTAAGA AACATCCGCC
 201 ACGTTGGCAA AATGGTTTCA CAACAGCACT TAGCATTGTG ATTACATTCC
 251 ACTTTGTAAC ATTTGGCTTT TTAATCTTCT CAGGTAAACT TATATAATAA
 301 AGGAGAATTT AATTATGGAA TTTAGAGAAC AAGTATTAAA TTTATTAGCA
 351 GAAGTAGCAG AAAAATGATA TTGTAAAAGA AAATCCAGAC GTAGAAATTT
 401 TTGAAGAAGG TATTATTGAT TCTTTCCAAA CAGTTGGATT ATTATTAGAG
 451 ATTCAAAATA AACTTGATAT CGAAGTATCT ATTATGGACT TTGATAGAAG
 501 ATGAGTGGGC MACACCAAAT AAAATCGTTG AAGCATTAGA AGAGTTACGA
 551 TGAAATTAAA ACCTTTTTTA CCCATTTTAA TTAGTGGAGC GGTATTCATT
 601 GTCTTTCTAT TATTACCTGC TAGTTGGTTT ACAGGATTAG TAAATGAAAA
 651 GACTGTAGAA GATAATAGAA CTTCATTGAC AGATCAAGTA CTAAAAGGCA
 701 CACTCAWTCA AGATAAGTTA TACGAATCAA ACAAGTATTA TCCTATATAC
 751 GGCTCTAGTG AATTAGGTAA AGATGACCCA TTTAATCCTG CAATTGCATT
 801 AAATAAGCAT AACGCCAACA AAAAAGCATT CTTATTAGGT GCTGGTGGTT
 851 CTACAGACTT AATTAACGCA GTTGAACTTG CATCACAGTT ATGATAAATT
 901 AAAAGGTTAA GAAATTAACA TTTATTATTT CACCACAATG GTTTACAAAC
 951 CCATGGTTTA ACGAATCCAA AACTTTGATG CTCSTATGTC TCAAACTCMA
1001 ATTAATCAAA TGTTCCCASC AGAAAAACAT GTCTACTGAA TTAAAACGTC
1051 GTTATGCACA ACGTTTATTA CAGTTTCCAC ATGTACACAA TAAAGAATAC
1101 TTGAAATCTT ATGCTAAAAA CCCTAAAGAA ACTAAAGRTA GTTATATTTC
1151 TGGKTTTWAA RAGAGATCAA TTGATTAAAA TAGAAGCGAT TAAATCATTG
1201 TTTGCAATGG ATAAATCTCC ATTAGAACAT GTTAAACCCT GCTACAAAAC
1251 CAGACGCTTC TTGGGATGAG ATGAAACAAA AAGCAGTTGA AATTGGTAAA
1301 GCTGATACTA CATCGAATAA ATTTGGTATT AGAGATCAAT ACTGGAAATT
1351 AATTCCAAGA AAGTAAGCCG TTAAAGTTAG ACGTTGACTA CGAATTCMAT
1401 GTTWATTCTC CCAGAATTCC MAGATTTAGA ATTACTTGTW AAAAMMATGC
1451 KTGCTGCTGG TGCAGATGTT CAATATGTAA GTATTCCATC AAACGGTGTA
1501 TGGTATGACC ACATTGGTAT CGATAAAGAA CGTCGTCAAG CAGTTTATAA
1551 AAAAATCCAT TCTACTGTTG TAGATAATGG TGGTAAAATT TACGATATGA
1601 CTGATAAAGA TTATGAAAAA TATGTTATCA GTGATGCCGT ACACATCGGT
1651 TGGAAAGGTT GGGTTTATAT GGATGAGCAA ATTGCGAAAC ATATGAAAGG
1701 TGAACCACAA CCTGAAGTAG ATAAACCTAA AAATTAAAAT ACAAATAGCA
1751 CATAACTCAA CGATTTTGAT TGAGCGTATG TGCTATTTTT ATATTTTAAA
1801 TTTCATAGAA TAGAATAGTA ATATGTGCTT GGATATGTGG CAATAATAAA
1851 ATAATTAATC AGATAAATAG TATAAAATAA CTTTCCCATC AGTCCAATTT
1901 GACAGCGAAA AAAGACAGGT AATAACTGAT TATAAATAAT TCAGTATTCC
1951 TGTCTTTGTT GTTATTCATA ATATGTTCTG TTAACTTAAT ATCTTTATAT
2001 TAGAATACTT GTTCTACTTC TATTACACCA GGCACTTCTT CGTGTAATGC
2051 ACGCTCAATA CCAGCTTTAA GAGTGATTGT AGAACTTGGG CATGTACCAC
2101 ATGCACCATG TAATTGTAAT TTAACAATAC CGTCTTCCAC GTCAATCAAT
2151 GAGCAGTCGC CACCATCACG TAATAAAAAT GGACGAAGAC GTTCAATAAC
2201 TTCTGCTACT TGATCGACCT GCAGGCATGC AAGC
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Mutant: NT166

Phenotype: temperature sensitivity

Sequence map: Mutant NT166 is complemented by plasmid pMP425, which carries a 3.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 66. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *nrdE*, encoding ribonucleotide diphosphate reductase II (EC 1.17.4.1), from *B. subtilis*(Genbank Accession No. Z68500), and *ymaA*, a hypothetical ORF, from *B. subtilis* (same Genbank entry).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP425, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP425

SEQ ID NO. 76

pMP425 Length: 3305 nt

```
  1 GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GATCCAATGA AAATAATATA
 51 TTTTTCATTT ACTGGAAATG TCCGTCGTTT TATTAAGAGA ACAGAACTTG
101 AAAATACGCT TGAGATTACA GCAGAAAATT GTATGGAACC AGTTCATGAA
151 CCGTTTATTA TCGTTACTGG CACTATTGGA TTTGGAGAAG TACCAGAACC
201 CGTTCAATCT TTTTTAGAAG TTAATCATCA ATACATCAGA GGTGTGGCAG
251 CTAGCGGTAA TCGAAATTGG GGACTAAATT TCGCAAAAGC GGGTCGCACG
301 ATATCAGAAG AGTATAATGT CCCTTTATTA ATGAAGTTTG AGTTACATGG
351 GAAAAAACAA AGACGTTATT GAATTTAAGA ACAAGGTGGG TAATTTTAAT
401 GAAAACCATG GAAGAGAAAA AGTACAATCA TATTGAATTA AATAATGAGG
451 TCACTAAACG AAGAGAAGAT GGATTCTTTA GTTTAGAAAA AGACCAAGAA
501 GCTTTAGTAG CTTATTTAGA AGAAGTAAAA GACAAAACAA TCTTCTTCGA
551 CACTGAAATC GAGCGTWTAC GTTMTTTAGT AGACMACGAT TTTTATTTCA
601 ATGTGTTTGA TATWTATAGT GAAGCGGATC TAATTGAAAT CACTGATTAT
651 GCAAAATCAA TCCCGTTTAA TTTTGCAAGT TATATGTCAG CTAGTAAATT
701 TTTCAAAGAT TACGCTTTGA AAACAAATGA TAAAAGTCAA TACTTAGAAG
751 ACTATAATCA ACACGTTGCC ATTGTTGCTT TATACCTAGC AAATGGTAAT
801 AAAGCACAAG CTAAACAATT TATTTCTGCT ATGGTTGAAC AAAGATATCA
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851 ACCAGCGACA CCAACATTTT TAAACGCAGG CCGTGCGCGT TCGTGGTGGA
901 GCTAGTGTTC ATTGTTTCCT TATTAGAAGT TGGATGGACA GCTTAAATTC
951 AATTTAACTT TATTGGATTC AACTGCAAAA CAATTAAGTW AAATTGGGGG
1001 CGGSGTTTGC MATTAACTTA TCTAAATTGC GTGCACGTGG TGAAGCAATT
1051 AAAGGAATTA AAGGCGTAGC GAAAGGCGTT TTACCTATTG CTAAGTCACT
1101 TGAAGGTGGC TTTAGCTATG CAGATCAACT TGGTCAACGC CCTGGTGCTG
1151 GTGCTGTGTA CTTAAATATC TTCCATTATG ATGTAGAAGA ATTTTTAGAT
1201 ACTAAAAAAG TAAATGCGGA TGAAGATTTA CGTTTATCTA CAATATCAAC
1251 TGGTTTAATT GTTCCATCTA AATTCTTCGA TTTAGCTAAA GAAGGTAAGG
1301 ACTTTTATAT GTTTGCACCT CATACAGTTA AAGAAGAATA TGGTGTGACA
1351 TTAGACGATA TCGATTTAGA AAAATATTAT GATGACATGG TTGCAAACCC
1401 AAATGTTGAG AAAAAGAAAA AGAATGCGCG TGAAATGTTG AATTTAATTG
1451 CGCMAACACA ATTACAATCA GGTTATCCAT ATTTAATGTT TAAAGATAAT
1501 GCTAACAGAG TGCATCCGAA TTCAAACATT GGACAAATTA AAATGAGTAA
1551 CTTATGTACG GAAATTTTCC AACTACAAGA AACTTCAATT ATTAATGACT
1601 ATGGTATTGA AGACGAAATT AAACGTGATA TTTCTTGTAA CTTGGGCTCA
1651 TTAAATATTG TTAATGTAAT GGAAAGCGGA AAATTCAGAG ATTCAGTTCA
1701 CTCTGGTATG GACGCATTAA CTGTTGTGAG TGATGTAGCA AATATTCAAA
1751 ATGCACCAGG AGTTAGAAAA GCTAACAGTG AATTACATTC AGTTGKTCTT
1801 GGGTGTGATG AATTWACACG GTTACCTAGC AAAAAATAAA ATTGGTTATG
1851 AGTCAGAAGA AGCAAAAGAT TTTGCAAATA TCTTCTTTAT GATGATGAAT
1901 TTCTACTCAA TCGAACGTTC AATGGAAATC GCTAAAGAGC GTGGTATCAA
1951 ATATCAAGAC TTTGAAAAGT CTGATTATGC TAATGGCAAA TATTTCGAGT
2001 TCTATACAAC TCAAGAATTT GAACCTCAAT TCGAAAAAGT ACGTGAATTA
2051 TTCGATGGTA TGGCTATTCC TACTTCTGAG GATTGGAAGA AACTACAACA
2101 AGATGTTGAA CAATATGGTT TATATCATGC ATATAGATTA GCAATTGCTC
2151 CAACACAAAG TATTTCTTAT GTTCAAAATG CAACAAGTTC TGTAATGCCA
2201 ATCGTTGACC AAATTGAACG TCGTACTTAT GGTAAATGCG GAAACATTTT
2251 ACCCTATGCC ATTCTTATCA CCACAAACAA TGTGGTACTA CAAATCAGCA
2301 TTCAATACTG ATCAGATGAA ATTAATCGAT TTAATTGCGA CAATTCAAAC
2351 GCATATTGAC CAAGGTATCT CAACGATCCT TTATGTTAAT TCTGAAATTT
2401 CTACACGTGA GTTAGCAAGA TTATATGTAT ATGCGCACTA TAAAGGATTA
2451 AAATCACTTT ACTATACTAG AAATAAATTA TTAAGTGTAG AAGAATGTAC
2501 AAGTTGTTCT ATCTAACAAT TAAATGTTGA AAATGACAAA CAGCTAATCA
2551 TCTGGTCTGA ATTAGCAGAT GATTAGACTG CTATGTCTGT ATTTGTCAAT
2601 TATTGAGTAA CATTACAGGA GGAAATTATA TTCATGATAG CTGTTAATTG
2651 GAACACACAA GAAGATATGA CGAATATGTT TTGGAGACAA AATATATCTC
2701 AAATGTGGGT TGAAACAGAA TTTAAAGTAT CAAAAGACAT TGCAAGTTGG
2751 AAGACTTTAT CTGAAGCTGA ACAAGACACA TTTAAAAAAG CATTAGCTGG
2801 TTTAACAGGC TTAGATACAC ATCAAGCAGA TGATGGCATG CCTTTAGTTA
2851 TGCTACATAC GACTGACTTA AGGAAAAAAG CAGTTTATTC ATTTATGGCG
2901 ATGATGGAGC AAATACACGC GAAAAGCTAT TCACATATTT TCACAACACT
2951 ATTACCATCT AGTGAAACAA ACTACCTATT AGATGAATGG GTTTTAGAGG
3001 AACCCCATTT AAAATATAAA TCTGATAAAA TTGTTGCTAA TTATCACAAA
3051 CTTTGGGGTA AAGAAGCTTC GATATACGAC CAATATATGG CCAGAGTTAC
3101 GAGTGTATTT TTAGAAACAT TCTTATTCTT CTCAGGTTTC TATTATCCAC
3151 TATATCTTGC TGGTCAAGGG AAAATGACGA CATCAGGTGA AATCATTCGT
3201 AAAATTCTTT TAGATGAATC TATTCATGGT GTATTTACCG GTTTAGATGC
3251 ACAGCATTTA CGAAATGAAC TATCTGAAAG TGAGAAACAA AAAGCAGATC
3301 GACCT

Mutant: NT 199

Phenotype: temperature sensitivity

Sequence map: Mutant NT199 is complemented by plasmid pMP642, which carries a 3.6 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 67. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *yybQ*, an uncharacterized ORFs identified in *B. subtilis* from genomic sequencing efforts.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP642, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP642

SEQ ID NO. 77

pMP642 Length: 1945 nt

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  1 TTGATAGTTT ATTGGAGAGA AAGAAGTATT AATCAAGTCG AAATCGTTGG
 51 TGTATGTACC GATATTTGCG TGTTACATAC AGCAATTTCT GCATACAACT
101 TAGGTTATAA AATTTCAGTA CCTGCTGAGG GAGTGGCTTC ATTTAATCAA
151 AAAGGGCATG AATGGGCACT TGCACATTTC AAAAACTCAT TAGGTGCAGA
201 GGTAGAACAA CACGTTTAAA TCGTGCTAAA ATAATTATAA AGAATACAAT
251 TTACAAGGGA GATATTTGAC AATGGCTAAA ACATATATTT TCGGACATAA
301 GAATCCAGAC ACTGATGCAA TTTCATCTGC GATTATTATG GCAGAATTTG
351 AACAACTTCG AGGTAATTCA GGAGCCAAAG CATACCGTTT AGGTGATGTG
401 AGTGCARAAA CTCAATTCGC GTTAGATACA TTTAATGTAC CTGCTCCGGA
451 ATTATTAACA GATGATTTAG ATGGTCAAGA TGTTATCTTA GTTGATCATA
501 ACGAATTCCA ACAAAGTTCT GATACGATTG CCTCTGCTAC AATTAAGCAT
551 GTAATTGATC ATCACAGAAT TGCAAATTTC GAAACTGCTG GTCCTTTATG
601 TTATCGTGCT GAACCAGTTG GTTGTACAGC TACAATTTTA TACAAAATGT
651 TTAGAGAACG TGGCTTTGAA ATTAAACCTG AAATTGCCGG TTTAATGTTA
701 TCAGCAATTA TCTCAGATAG CTTACTTTTC AAATCACAAC ATGTACACAA
751 CAAGATGTTA AAGCAGCTGA AGAATTAAAA GATATTGCTA AAGTTGATAT
801 TCAAAAGTAC GGCTTAGATA TGTTAAAAGC AGGTGCTTCA ACAACTGATA
851 AATCAGTTGA ATTCTTATTA AACATGGATG CTAAATCATT TACTATGGGT
901 GACTATGKGA YTCGTATTGC AACAAGTTAA TGCTGTTGAC CTTGACGAAG
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 951 TGTTAAWTCG TAAAGAAGAT TTAGAAAAAG AAATGTTAGC TGTAAGTGCA
1001 CAAGAAAAAT ATGACTTATT TGTACTTGTT GTTACKGACA TCATTAATAG
1051 TGATTCTAAA ATTTTAGTTG TAGGTGCTGA AAAAGATAAA GTTGGCGAAG
1101 CATTCAATGT TCAATTAGAA GATGACATGG CCYTCTTATC TGGTGTCGTW
1151 TCTCGAAAAA AACAAATCGT ACCTCAAATC ACTGAAGCAT TAACAAAATA
1201 ATACTATATT ACTGTCTAAT TATAGACATG TTGTATTTAA CTAACAGTTC
1251 ATTAAAGTAG AATTTATTTC ACTTTCCAAT GAACTGTTTT TTATTTACGT
1301 TTGACTAATT TACAACCCTT TTTCAATAGT AGTTTTTATT CCTTTAGCTA
1351 CCCTAACCCA CAGATTAGTG ATTTCTATAC AATTCCCCTT TTGTCTTAAC
1401 ATTTTCTTAA AATATTTGCG ATGTTGAGTA TAAATTTTTG TTTTCTTCCT
1451 ACCTTTTTCG TTATGATTAA AGTTATAAAT ATTATTATGT ACACGATTCA
1501 TCGCTCTATT TTCAACTTTC AACATATATA ATTCGAAAGA CCATTTAAAA
1551 TTAACGGCCA CAACATTCAA ATCAATTAAT CGCTTTTTCC AAAATAATCA
1601 TATAAGGAGG TTCTTTTCAT TATGAATATC ATTGAGCAAA AATTTTATGA
1651 CAGTAAAGCT TTTTTCAATA CACAACAAAC TAAAGATATT AGTTTTAGAA
1701 AAGAGCAATT AAAGAAGTTA AGCAAAGCTA TTAAATCATA CGAGAGCGAT
1751 ATTTTAGAAG CACTATATAC AGATTTAGGA AAAAATAAAG TCGAAGCTTA
1801 TGCTACTGAA ATTGGCATAA CTTTGAAAAG TATCAAAATT GCCCGTAAGG
1851 AACTTAAAAA CTGGACTAAA ACAAAAAATG TAGACACACC TTTATATTTA
1901 TTTCCAACAA AAAGCTATAT CAAAAAAGAA CCTTATGGAA CAGTT
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Mutant: NT 201

Phenotype: temperature sensitivity

Sequence map: Mutant NT201 is complemented by plasmid pMP269, which carries a 2.6 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 68. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarity to *ylxC*, encoding a putative *murB* homolog (UDP-N-acetylenolpyruvoylglucosamine reductase), in *B. subtilis* (Genbank Accession No. M31827). The predicted relative size and orientation of the *ylxC* gene is depicted by an arrow in the map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP269, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP269

SEQ ID NO. 78

pMP269 Length: 2590 nt

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   1 TCGAACTCGG TACCCGGGGA TCCTCTAGAG TCGATCAACT ACAACTACAA
  51 TTAAACAAAT TGAGGAACTT GATAAAGTTG TAAAATAATT TTAAAAGAGG
 101 GGAACAATGG TTAAAGGTCT TAATCATTGC TCCCCTCTTT TCTTTAAAAA
 151 AGGAAATCTG GGACGTCAAT CAATGTCCTA GACTCTAAAA TGTTCTGTTG
 201 TCAGTCGTTG GTTGAATGAA CATGTACTTG TAACAAGTTC ATTTCAATAC
 251 TAGTGGGCTC CAAACATAGA GAAATTTGAT TTTCAATTTC TACTGACAAT
 301 GCAAGTTGGC GGGGCCCAAA CATAGAGAAT TTCAAAAAGG AATTCTACAG
 351 AAGTGGTGCT TTATCATGTC TGACCCACTC CCTATAATGT TTTGACTATG
 401 TTGTTTAAAT TTCAAAATAA ATATGATAGT GATATTTACA GCGATTGTTA
 451 AACCGAGATT GGCAATTTGG ACAACGCTCT ACCATCATAT ATTCATTGAT
 501 TGTTAATTCG TGTTTGCATA CACCGCATAA GATTGCTTTT TCGTTAAATG
 551 AAGGCTCAGA CCAACGCTTA ATGGCGTGCT TTTCAAACTC ATTATGGCAC
 601 TTATAGCATG GATAGTATTT ATTACAACAT TTAAATTTAA TAGCAATAAT
 651 ATCTTCTTCG GTAAAATAAT GGCGACAGCG TGTTTCAGTA TCGATTAATG
 701 AACCATAAAC TTTAGGCATA GACAAAGCTC CTTAACTTAC GATTCCTTTG
 751 GATGTTCACC AATAATGCGA ACTTCACGAT TTAATTCAAT GCCAAWTTTT
 801 TCTTTGACGG TCTTTTGTAC ATAATGAATA AGGTTTTCAT AATCTGTAGC
 851 AGTTCCATTG TCTACATTTA CCATAAAACC AGCGTGTTTG GTTGAAACTT
 901 CAACGCCGCC AATACGGTGA CCTTGCAAAT TAGAATCTTG TATCAATTTA
 951 CCTGCAAAAT GACCAGGCGG TCTTTGGAAT ACACTACCAC ATGAAGGATA
1001 CTCTAAAGGT TGTTTAAATT CTCTACGTTC TGTTAAATCA TCCATTTTAG
1051 CTTGTATTTC AGTCATTTTA CCAGGAGCTA AAGTAAATGC AGCTTCTAAT
1101 ACAACTAANT GTTCTTTTTG AATAATGCTA TTACNATAAT CTAACTCTAA
1151 TTCTTTTGTT GTAAGTTTAA TTAACGAGCC TTGTTCGTTT ACGCAAAGCG
1201 CATRGTCTAT ACAATCTTTA ACTTCGCCAC CATAAGCGCC AGCATTCATA
1251 TACACTGCAC CACCAATTGA ACCTGGAATA CCACATGCAA ATTCAAGGCC
1301 AGTAAGTGCG TAATCACGAG CAACACGTGA GACATCAATA ATTGCAGCGC
1351 CGCTACCGGC TATTATCGCA TCATCAGATA CTTCCGATAT GATCTAGTGA
1401 TAATAAACTA ATTACAATAC CGCGAATACC ACCTTCACGG ATAATAATAT
1451 TTGAGCCATT TCCTAAATAT GTAACAGGAA TCTCATTTTG ATAGGCATAT
1501 TTAACAACTG CTTGTACTTC TTCATTTTTA GTAGGGGTAA TGTAAAAGTC
1551 GGCATTACCA CCTGTTTTAG TATAAGTGTA TCGTTTTAAA GGTTCATCAA
1601 CTTTAATTTT TTCAKTYGRS MTRARKKSWT GYAAAGCTTG ATAGATGTCT
1651 TTATTTATCA CTTCTCAGTA CATCCTTTCT CATGTCTTTA ATATCATATA
1701 GTATTATACC AATTTTAAAA TTCATTTGCG AAAATTGAAA AGRAAGTATT
1751 AGAATTAGTA TAATTATAAA ATACGGCATT ATTGTCGTTA TAAGTATTTT
1801 TTACATAGTT TTTCAAAGTA TTGTTGCTTT TGCATCTCAT ATTGTCTAAT
1851 TGTTAAGCTA TGTTGCAATA TTTGGTGTTT TTTTGTATTG AATTGCAAAG
1901 CAATATCATC ATTAGTTGAT AAGAGGTAAT CAAGTGCAAG ATAAGATTCA
1951 AATGTTTGGG TATTCATTTG AATGATATGT AGACGCACCT GTTGTTTTAG
2001 TTCATGAAAA TTGTTAAACT TCGCCATCAT AACTTTCTTA GTATATTTAT
2051 GATGCAAACG ATAAAACCCT ACATAATTTA AGCGTTTTTC ATCTAAGGAT
2101 GTAATATCAT GCAAATTTTC TACACCTACT AAAATATCTA AAATTGGCTC
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2151  TGTTGAATAT TTAAAATGAT GCGTACCGCC AATATGTTTT GTATATTTTA
2201  CTGGGCTGTC TAAGAGGTTG AATAATAATG ATTCAATTTC AGTGTATTGT
2251  GATTGAAAAC AATTAGTTAA ATCACTATTA ATGAATGGTT GAACATTTGA
2301  ATACATGATA AACTCCTTTG ATATTGAAAA TTAATTTAAT CACGATAAAG
2351  TCTGGAATAC TATAACATAA TTCATTTTCA TAATAAACAT GTTTTTGTAT
2401  AATGAATCTG TTAAGGAGTG CAATCATGAA AAAAATTGTT ATTATCGCTG
2451  TTTTAGCGAT TTTATTTGTA GTAATAAGTG CTTGTGGTAA TAAAGAAAAA
2501  GAGGCACAAC ATCMATTTAC TAAGCAATTT AAAGATGTTG AGCAAACACA
2551  WAAAGAATTA CAACATGTCA TGGATAATAT ACATTTGAAA
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Mutant: NT304

Phenotype: temperature sensitivity

Sequence map: Mutant NT304 is complemented by plasmid pMP450, which carries a 3.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 69. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities from the left-most contig below and the *dod* gene product, encoding pentose-5-phosphate epimerase (EC 5.1.3.1), from *S. oleraceae* (Genbank Accession No. L42328).

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP450, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP450

SEQ ID NO. 79

pMP450.forward Length: 1019 nt

```
  1  ATTCGAGCTC GGTACCCGGG GATCCTCTAG AGTCGCTCGA TAACTTCTAT
 51  ATGAACATCA TGTTTATAAT ATGCTTTTTT CAATAATAAC TGAATTGCCC
101  CAAAAAAGTG ATCTAATCGT CCGCCTGTTG CACCATAAAT TGTAATACTA
151  TCAAATCCAA GTGCAACAGC TTTATCAACC GCTAAAGCTA AATCCGTATC
201  AGCTTTTTCA GCTTGAACTG GTTTGATTTG TAACTGTTCT GTTAGAAGTT
251  GGCGTTCTTC TTTACTGACT GAATCAAAGT CTCCCACTGA GAAAAAAGGG
301  ATAATTTGAT GCTTCAATAA AATCAAAGCA CCTCTATCAA CGCCGCCCCA
351  TTTACCTTCA TTACTTTTGG CCCAAATATC TTGCGGCAAG TGTCGATCAG
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 401  AACATAATAA ATTTATATGC ATATACACTC AACCTTTCAA TGCTTGTGTT
 451  GACTTTTTTA TAATCCTCTT GTTTAAAGAA AAATGAACCT GTTACTAGCA
 501  TTGTTAGCAC CATTTTCAAC ACAAACTTTC GCTGTTATCG GTATTTACGC
 551  CTCCATCAAC TTCAATATCA AAGTTTAATT GACGTTCCAT TTTAATAGCA
 601  TTAAGACCCG CTATTTTTTC TACGCATTGA TCAATAAATG ATTGACCACC
 651  AAACCCTGGG TTAACTGTCA TCACTAGTAC ATAATCAACA ATGTCTAAAA
 701  TAGGTTCAAT TTGTGATATT GGTGTACCAG GATTAATTAC TACACCAGCT
 751  TTTTTATCTA AATGTTTAAT CATTTGAATA GCACGATGAA ATATGAGGCG
 801  TTGATTCGAC ATGAATTGNA AATCATATCG GCACCATGTT CTGCAAATGA
 851  TGCAATATAC TTTTCTGGAA TTTTCAATCA TCAAATGTAC GTCTATANGT
 901  AATGTTGTGC CTTTTCTTAC TGCATCTAAT ATTGGTAAAC CAATAGATAT
 951  ATTAGGGACA AATTGACCAT CCATAACATC AAAATGAACT CCGTCGAANC
1001  CCGGCTTCTC CAGTCGTTT
```

SEQ ID NO. 80

pMP450.reverse Length: 1105 nt

```
   1  CNTGCATGCC TGCAGGTCGA TCTANCAAAG CATATTAGTG AACATAAGTC
  51  GAATCAACCT AAACGTGAAA CGACGCAAGT ACCTATTGTA AATGGGCCTG
 101  CTCATCATCA GCAATTCCAA AAGCCAGAAG GTACGGTGTA CGAACCAAAA
 151  CCTAAAAAGA AATCAACACG AAAGATTGTG CTCTTATCAC TAATCTTTTC
 201  GTTGTTAATG ATTGCACTTG TTTCTTTTGT GGCAATGGCA ATGTTTGGTA
 251  ATAAATACGA AGAGACACCT GATGTAATCG GGAAATCTGT AAAAGAAGCA
 301  GAGCAAATAT TCAATAAAAA CAACCTGAAA TTGGGTAAAA TTTCTAGAAG
 351  TTATAGTGAT AAATATCCTG AAAATGAAAT TATTAAGACA ACTCCTAATA
 401  CTGGTGAACG TGTTGAACGT GGTGACAGTG TTGATGTTGT TATATCAAAG
 451  GGSCCTGAAA AGGTTAAAAT GCCAAATGTC ATTGGTTTAC CTAAGGAGGA
 501  AGCCTTGCAG AAATTAAAAT CCGTTAGGTC TTAAAGATGT TACGATTGAA
 551  AAAGTWTATA ATAATCCAAG CGCCMAAAGG ATACATTGCA AATCAAAKTG
 601  TTAMCCGCAA ATACTGAAAT CGCTATTCAT GATTCTAATA TTAAACTATA
 651  TGAATCTTTA GGCATTAAGC AAGTTTATGT AGAAGACTTT GAACATAAAT
 701  CCTTTAGCAA AGCTAAAAAA GCCTTAGAAG AAAAAGGGTT TAAAGTTGAA
 751  AGTAAGGAAG AGTATAGTGA CGATATTGAT GAGGGTGATG TGATTTCTCA
 801  ATCTCCTAAA GGAAAATCAG TAGATGAGGG GTCAACGATT TCATTTGTTG
 851  TTTCTAAAGG TAAAAAAAGT GACTCATCAG ATGTCNAAAC GACAACTGAA
 901  TCGGTAGATG TTCCATACAC TGGTNAAAAT GATAAGTCAC AAAAAGTTCT
 951  GGTTTATCTT NAAGATAANG ATAATGACGG TTCCACTGAA AAAGGTAGTT
1001  TCGATATTAC TAATGATCAC GTTATAGACA TCCTTTAAGA ATTGAAAAAG
1051  GGAAAACGCA GTTTTATTGT TAAATTGACG GTAAACTGTA CTGAAAAAAA
1101  NTCGC
```

Mutant: NT 310

Phenotype: temperature sensitivity

Sequence map: Mutant NT310 is complemented by plasmid pMP364, which carries a 2.4 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted

in Fig. 70; there are no apparent restriction sites for EcoR I, BamH I, HinD III or Pst I. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to the *ddlA* gene product from *E. hirae*, which encodes D-Ala-D-Ala ligase (EC 6.3.2.4); similarities are also noted to the functionally-similar proteins VanA and VanB from *E. faecium* and the VanC protein from *E. gallinarum*. The predicted relative size and orientation of the *ddlA* gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP364, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP364

SEQ ID NO. 81
pMP364 Length: 2375 nt

```
   1 AATATGACAG AACCGATAAA GCCAAGTTCC TCTCCAATCA CTGAAAAGAT
  51 AAAGTCAGTA TGATTTTCAG GTATATAAAC TTCACCGTGA TTGTATCCTT
 101 TACCTAGTAA CTGTCCAGAA CCGATAGCTT TAAGTGATTC AGTTAAATGA
 151 TAGCCATCAC CACTACTATA TGTATAGGGG TCAAGCCATG AATTGATTCG
 201 TCCCATTTGA TACAGTTGGA CACCTAATAA ATTTTCAATT AATGCGGGTG
 251 CATATAGAAT ACCTAAAATG ACTGTCATTG CACCAACAAT ACCTGTAATA
 301 AAGATAGGTG CTAAGATACG CCATGTTATA CCACTTACTA ACATCACACC
 351 TGCAATAATA GCAGCTAATA CTAATGTAGT TCCTAGGTCA TTTTGCAGTA
 401 ATATTAAAAT ACTTGGTACT AACGAGACAC CAATAATTTT GAAAAATAAT
 451 AACAAATCAC TTTGGAATGA TTTATTGAAT GTGAATTGAT TATGTCTAGA
 501 AACGACACGC GCTAATGCTA AAATTAAAAT AATTTTCATG AATTCAGATG
 551 GCTGAATACT GATAGGGCCA AACGTGTTYC AACTTTTGGC ACCATTGATA
 601 ATAGGTGTTA TAGGTGACTC AGGAATAACG AACCAGCCTA TTWATAWTAG
 651 ACAGATTAAG AAATACAATA AATATGTATA ATGTTTAATC TTTTTAGGTG
 701 AAATAAACAT GATGATACCT GCAAAAATTG CACCTAAAAT GTAATAAAAA
 751 ATTTGTCTGA TACCGAAATT AGCACTGTAT TGACCACCGC CCATTGCCGA
 801 GTTAATAAGC AGAACACTGA AAATTGCTAA AACAGCTATA GTGGCTACTA
 851 ATACCCAGTC TACTTTGCGA AGCCAATGCT TATCCGGCTG TTGACGAGAT
 901 GAATAATTCA TTGCAAACTC CTTTTATACT CACTAATGTT TATATCAATT
 951 TTACATGACT TTTTAAAAAT TAGCTAGAAT ATCACAGTGA TATCAGCYAT
1001 AGATTTCAAT TTGAATTAGG AATAAAATAG AAGGGAATAT TGTTCTGATT
1051 ATAAATGAAT CAACATAGAT ACAGACACAT AAGTCCTCGT TTTTAAAATG
```

1101 CAAAATAGCA TTAAAATGTG ATACTATTAA GATTCAAAGA TGCGAATAAA
1151 TCAATTAACA ATAGGACTAA ATCAATATTA ATTTATATTA AGGTAGCAAA
1201 CCCTGATATA TCATTGGAGG GAAAACGAAA TGACAAAAGA AAATATTTGT
1251 ATCGTTTTTG GAGGGAAAAG TGCAGAACAC GAAGTATCGA TTCTGACAGC
1301 AYWAAATGTA TTAAATGCAR TAGATAAAGA CAAATATCAT GTTGATATCA
1351 TTTATATTAC CAATGATGGT GATTGGAGAA AGCAAAATAA TATTACAGCT
1401 GAAATTAAAT CTACTGATGA GCTTCATTTA GAAAAATGGA GAGGCGCTTG
1451 AGATTTCACA GCTATTGAAA GAAAGTAGTT CAGGACAACC ATACGATGCA
1501 GTATTCCCAT TATTACATGG TCCTAATGGT GAAGATGGCA CGATTCAAGG
1551 GCTTTTTGAA GTTTTGGATG TACCATATGT AGGAAATGGT GTATTGTCAG
1601 CTGCAAGTTT CTATGGACAA ACTTGTAATG AAACAATTAT TTGAACATCG
1651 AGGGTTACCA CAGTTACCTT ATATTAGTTT CTTACGTTCT GAATATGAAA
1701 AATATGAACA TAACATTTTA AAATTAGTAA ATGATAAATT AAATTACCCA
1751 GTCTTTGTTA AACCTGCTAA CTTAGGGTCA AGTGTAGGTA TCAGTAAATG
1801 TAATAATGAA GCGGAACTTA AAGGAGGTAT TAAAGAAGCA TTCCAATTTG
1851 ACCGTAAGCT TGTTATAGAA CAAGGCGTTA ACGCAACGTG AAATTGAAGT
1901 AGCAGTTTTA GGAAATGACT ATCCTGAAGC GACATGGCCA GGTGAAGTCG
1951 TAAAAGATGT CGCGTTTTAC GATTACAAAT CAAAATATAA AGGATGGTAA
2001 GGTTCAATTA CAAATTCCAG CTGACTTAGA CGGAAGATGT TCAATTAACG
2051 GCTTAGAAAT ATGGCATTAG AGGCATTCAA AGCGACAGAT TGTTCTGGTT
2101 TAGTCCGTGC TGATTTCTTT GTAACAGAAG ACAACCAAAT ATATATTAAT
2151 GAAACAAATG CAATGCCTGG ATTTACGGCT TTCAGTATGT ATCCAAAGTT
2201 ATGGGAAAAT ATGGGCTTAT CTTATCCAGA ATTGATTACA AAACTTATCG
2251 AGCTTGCTAA AGAACGTCAC CAGGATAAAC AGAAAAATAA ATACAAAATT
2301 SMCTWAMTGA GGTTGTTATK RTGATTAAYG TKACMYTAWA GYAAAWTCAA
2351 TCATGGATTN CCTTGTGAAA TTGAA

Mutant: NT 312

Phenotype: temperature sensitivity

Sequence map: Mutant NT312 is complemented by plasmid pMP266, which carries a 1.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 71; there are no apparent restriction sites for EcoR I, BamH I, HinD III or Pst I. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *mg442*, a hypothetical ORF from *M. genetalium*, and limited similarities to G-proteins from human and rat clones; this probably indicates a functional domain of a new Staph. protein involved in GTP-binding. The ORF contained within clone pMP266 is novel and likely to be a good candidate for screen development.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP266, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP266

SEQ ID NO. 82

pMP266 Length: 1543 nt

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   1 AATCATTTTC AGTTTATCAT TAAACAAATA TATTGAACYM MYMAAAATGT
  51 CATACTGATA AAGATGAATG TCACTTAATA AGTAACTTAG ATTTAACAAA
 101 TGATGATTTT TAATTGTAGA AAACTTGAAA TAATCACTTA TACCTAAATC
 151 TAAAGCATTG TTAAGAAGTG TGACAATGTT AAAATAAATA TAGTTGAATT
 201 AATGAATTTG TTCTAYAATT AACAKGTTWT WGAWTTTAAT AATGAGAAAA
 251 GAATTGACGA AAGTAAGGTG AATTGAATGG TTATTCMATG GTATCCAGGA
 301 CMTATGGCGA AAAGCCAAAA GAGAAGTAAG TGAACAATTA AMAAAAGTAG
 351 ATGTAGTGTT TGAACTAGTA GATGCAAGAA TTCCATATAG TTCAAGAAAC
 401 CCTATGATAG ATGAAGTTAT TAACCAAAAA CCACGTGTTG TTATATTAAA
 451 TAAAAAAGAT ATGTCTAATT TAAATGAGAT GTCAAAATGG GAACAATTTT
 501 TTATTGATAA AGGATACTAT CCTGTATCAG TGGATGCTAA GCACGGTAAA
 551 AATTTAAAGA AAGTGGAAGC TGCAGCAATT AAGGCGACTG CTGAAAAATT
 601 TGAACGCGAA AAAGCGAAAG GACTTAAACC TAGAGCGATA AGAGCAATGA
 651 TCGTTGGAAT TCCAAATGTT GGTAAATCCA CATTAATAAA TAAACTGGCA
 701 AAGCGTAGTA TTGCGCAGAC TGGTAATAAA CCAGGTGTGA CCAAACAACA
 751 ACAATGGATT AAAGTTGGTA ATGCATTACA ACTATTAGAC ACACCAGGGA
 801 TACTTTGGCC TAAATTTGAA GATGAAGAAG TCGGTAAGAA GTTGAGTTTA
 851 ACTGGTGCGA TAAAAGATAG TATTGTGCAC TTAGATGAAG TTGCCATCTA
 901 TGGATTAAAC TTTTTAATTC AAAATGATTT AGCGCGATTA AAGTCACATT
 951 ATAATATTGA AGTTCCTGAA GATGCMGAAA TCATAGCGTG GTTTGATGCG
1001 ATAGGGAAAA AACGTGGCTT AATTCGACGT GGTAATGAAA TTGATTACGA
1051 AGCAGTCATT GAACTGATTA TTTATGATAT TCGAAATGCT AAAATAGGAA
1101 ATTATTGTTT TGATATTTTT AAAGATATGA CTGAGGAATT AGCAAATGAC
1151 GCTAACAATT AAAGAAGTTA CGCAGTTGAT TAATGCGGTT AATACAATAG
1201 AAGAATTAGA AAATCATGAA TGCTTTTTAG ATGAGCGAAA AGGTGTTCAA
1251 AATGCCATAG CTAGGCGCAG AAAAGCGTTA GAAAAAGAAC AAGCTTTAAA
1301 AGAAAAGTAT GTTGAAATGA CTTACTTTGA AAATGAAATA TTAAAAGAGC
1351 ATCCTAATGC TATTATTTGT GGGATTGATG AAGTTGGAAG AGGACCTTTA
1401 GCAGGTCCAG TCGTTGCATG CGCAACAATT TTAAATTCAA ATCACAATTA
1451 TTTGGGCCTT GATGACTCGA AAAAAGTACC TGTTACGAAA CGTCTAGAAT
1501 TAAATGAAGC ACTAAAAAAT GAAGTTACTG YTTTTGCATA TGG
```

Mutant: NT 318

Phenotype: temperature sensitivity

Sequence map: Mutant NT318 is complemented by plasmid pMP270, which carries a 2.2 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 72; there are no apparent restriction sites for EcoR I, BamH I, HinD III, or Pst I. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to the *spoVC* gene from *B. subtilis*, a gene identified as being important in sporulation, and the *pth* gene from *E. coli*, which encodes aminoacyl-tRNA hydrolase (EC 3.1.1.29). It is highly likely that the *spoVC* and *pth* gene products are homologues and that the essential gene identified here is the Staph. equivalent. The predicted relative size and orientation of the *spoVC* gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP270, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP270

SEQ ID NO. 83

pMP270 Length: 2185 nt

```
  1 TTAAACAATT AAGAAAATCT GGTAAAGTAC CAGCASYAGT ATACGGTTAC
 51 GGTACTAAAA ACGTGTCAGT TAAAGTTGAT GAAGTAGAAT TCATCAAAGT
101 TATCCGTGAA GTAGGTCGTA ACGGTGTTAT CGAATTAGGC GTTGGTTCTA
151 AAACTATCAA AGTTATGGTT GCAGACTACC AATTCGATCC ACTTAAAAAC
201 CAAATTACTC ACATTGACTT CTTWKCAATC AATATGAGTG AAGAACGTAC
251 TGTTGAAGTA CCAGTTCAAT TAGTTGGTGA AGCAGTAGGC GCTAAAGAAA
301 GGCGGCGTTA GTTGAACAAC CATTATTCAA CTTAGAAAGT AACTGCTACT
351 CCAGACAATA TTCCAGAAGC AATCGAAGTA GACATTACTG AATTAAACAT
401 TAACGACAGC TTAACTGTTG CTGATGTTAA AGTAACTGGC GACTTCAAAA
451 TCGAAAACGA TTCAGCTGAA TCAGTAGTAA CAGTAGTTGC TCCAACTGAA
501 GAACCAACTG AAGAAGAAAT CGAAGCCTAT GGAAGGCGAA CAMCAAACTG
551 AAGAACCAGA AGTTGTTGGC GAAAGCAAAG AAGACGAAGA AAAAACTGAA
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601 GAGTAATTTT AATCTGTTAC ATTAAAGTTT TTATACTTTG TTTAACAAGC
651 ACTGTGCTTA TTTTAATATA AGCATGGTGC TTTTKGTGTT ATTATAAAGC
701 TTAATTAAAC TTTATWACTT TGTACTAAAG TTTAATTAAT TTTAGTGAGT
751 AAAAGACATT AAACTCAACA ATGATACATC ATAAAAATTT TAATGTACTC
801 GATTTTAAAA TACATACTTA CTAAGCTAAA GAATAATGAT AATTGATGGC
851 AATGGCGGAA AATGGATGTT GTCATTATAA TAATAAATGA AACAATTATG
901 TTGGAGGTAA ACACGCATGA AATGTATTGT AGGTCTAGGT AATATAGGTA
951 AACGTTTTGA ACTTACAAGA CATAATATCG GCTTTGAAGT CGTTGATTAT
1001 ATTTTAGAGA AAAATAATTT TTCATTAGAT AAACAAAAGT TTAAAGGTGC
1051 ATATACAATT GAACGAATGA ACGGCGATAA AGTGTTATTT ATCGAACCAA
1101 TGACAATGAT GAATTTGTCA GGTGAAGCAG TTGCACCGAT TATGGATTAT
1151 TACAATGTTA ATCCAGAAGA TTTAATTGTC TTATATGATG ATTTAGATTT
1201 AGAACAAGGA CAAGTTCGCT TAAGACAAAA AGGAAGTGCG GGCGGTCACA
1251 ATGGTATGAA ATCAATTATT AAAATGCTTG GTACAGACCA ATTTAAACGT
1301 ATTCGTATTG GTGTGGGAAG ACCAACGAAT GGTATGACGG TACCTGATTA
1351 TGTTTTACAA CGCTTTTCAA ATGATGAAAT GGTAACGATG GGAAAAAGTT
1401 ATCGAACACG CAGCACGCGC AATTGAAAAG TTTGTTGAAA CATCACRATT
1451 TGACCATGTT ATGAATGAAT TTAATGGTGA AKTGAAATAA TGACAATATT
1501 GACAMCSCTT ATAAAAGAAG ATAATCATTT TCAAGACCTT AATCAGGTAT
1551 TTGGACAAGC AAACACACTA GTAACTGGTC TTTCCCCGTC AGCTAAAGTG
1601 ACGATGATTG CTGAAAAATA TGCACAAAGT AATCAACAGT TATTATTAAT
1651 TACCAATAAT TTATACCAAG CAGATAAATT AGAAACAGAT TTACTTCAAT
1701 TTATAGATGC TGAAGAATTG TATAAGTATC CTGTGCAAGA TATTATGACC
1751 GAAGAGTTTT CAACACAAAG CCCTCAACTG ATGAGTGAAC GTATTAGAAC
1801 TTTAACTGCG TTAGCTCCAA GGTAAGAAAG GGTTATTTAT CGTTCCTTTA
1851 AATGGTTTGA AAAAGTGGTT AACTCCTGTT GAAATGTGGC AAAATCACCA
1901 AATGACATTG CGTGTTGGTG AGGATATCGA TGTGGACCAA TTTMWWAACA
1951 AATTAGTTAA TATGGGGTAC AAACGGGAAT CCGTGGTATC GCATATTGGT
2001 GAATTCTCAT TGCGAGGAGG TATTATCGAT ATCTTTCCGC TAATTGGGGA
2051 ACCAATCAGA ATTGAGCTAT TTGATACCGA AATTGATTCT ATTCGGGATT
2101 TTGATGTTGA AACGCAGCGT TCCAAAGATA ATGTTGAAGA AGTCGATATC
2151 ACAACTGCAA GTGATTATAT CATTACTGAA GAAGT

Mutant: NT 321
Phenotype: temperature sensitivity
Sequence map: Mutant NT321 is complemented by plasmid pMP276, which carries a 2.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 73; no apparent sites for HinD III, EcoR I, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to a hypothetical ORF of unknown function from *M. tuberculosis* (Genbank Accession No. Z73902).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP276, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP276

SEQ ID NO. 84

pMP276 Length: 2525 nt

1	AATCTGTTCC	TACTACAATA	CCTTGTCGGT	TTGAAGCACC	NGAAAATNGT
51	ACTTTCATAC	GTTCACGCGC	TTTTTCATTT	CCTTTTTGGA	AATCTGTAAG
101	AACAATACCG	GCTTCTTTTA	ATGATTGCAC	ACTTTGATCA	ACTGCAGGCT
151	TAATATTGAC	TGTTACTATT	TCATCTGGTT	CAATGAATCG	CAAAGCTTGC
201	TCAACTTCAT	CAGCATCTTT	TTGAACTCCA	TAAGGTAATT	TAACTGCAAT
251	AAACGTACAA	TCAATGCCTT	CTTCACGTAA	TTCGTTAACA	GACATTTGTA
301	CTAGTTTTCC	AACTAATGTA	GAATCCTGTC	CTCCTGAAAT	ACCTAACACT
351	AAAGATTTTA	TAAATGAATG	TGATTGTACA	TAATTTTTTA	TAAATTGCTT
401	TAATTCCATA	ATTTCTTCAG	CACTATCGAT	ACGCTTTTTC	ACTTTCATTT
451	CTTGTACAAT	AACGTCTTGT	AATTTACTCA	TTATCTTCTT	CCATCTCCTT
501	AACGTGTTCC	GCAACTTCAA	AAATACGTTT	ATGTTTATTA	TCCCAACATG
551	CCTTGCTTAA	ATCGACTGGA	TATTCTTGTG	GATTCAGGAA	ACGCTTATTT
601	TCATCCCAAA	TAGATTGTAA	TCCTAGTGCT	AAATATTCAC	GTGATTCATC
651	TTCTGTTGGC	ATTTGATATA	CTAATTTACC	ATTTTCATAA	ATATTATGAT
701	GCAAATCAAT	GGCTTCGAAA	GATTTTATAA	ATTTCATTTT	ATAAGTATGC
751	ACTGGATGGA	ATAATTTTAA	AGGTTGTTCA	TCGTATGGAT	TTTCATTTTC
801	CAAAGTAATA	TAATCGCCTT	CTGCCTTACC	TGTTTTCTTG	TTTATAATGC
851	GATATACATT	TTTCTTACCT	GGCGTCGTAA	CCTTTTCAGC	GTTATTTGAT
901	AATTTAATAC	GATCACTATA	TGAACCATCT	TCATTTTCAA	TAGCTACAAG
951	TTTATATACT	GCACCTAATG	CTGGTTGATC	GTATCCTGTA	ATCAGCTTTG
1001	TACCAACGCC	CCAAGAATCT	ACTTTTGCAC	CTTGTGCTTT	CAAACTCGTA
1051	TTCGTTTCTT	CATCCAAATC	ATTAGAYGCG	ATAATTTTAG	TTTCAGTAAA
1101	TCCTGYTTCA	TCAAGCATAC	GTCTTGCYTC	TTTAGATAAA	TAAGCGATAT
1151	CTCCAGAATC	TAATCGAATA	CCTAACAAAG	TTAATTTTGT	CACCTAATTC
1201	TTTTGCAACT	TTTATTGCAT	TTGGCACGCC	AGATTTTAAA	GTATGGAATG
1251	TATCTACTAG	GAACACACAA	TTTTTATGTC	TTTCAGCATA	TTTTTTGAAG
1301	GCAACATATT	CGTCTCCATA	AGTTTGGACA	AATGCATGTG	CATGTGTACC
1351	AGACACAGGT	ATACCAAATA	ATTTTCCCCG	CCCTAACATT	ACTTGTAGAA
1401	TCAAAGCCCC	CGATGTAAGC	AGCTCTAGCG	CCCCACAATG	CTGCATCAAT
1451	TTCTTGCGCA	CGACGTGTTA	CCAAACTCCA	TTAATTTATC	ATTTGATGCA
1501	ATTTGACGAA	ATTCTGCTAG	CCTTTGTTGT	AATTAATGTA	TGGAAATTTA
1551	CAATGTTTAA	TAAAATTGTT	CTATTAATTG	CGCTTGAATC	AATGGTGCTT
1601	CTACGCGTAA	CAATGGTTCG	TTACCAAAGC	ATAATTCGCC	TTCTTGCATC
1651	GAACGGATGC	TGCCTGTGAA	TTTTAAATCT	TTTAAATATG	ATAAGAAATC
1701	ATCCTTGTAG	CCAATAGACT	TTAAATATTC	CAAATCAGAT	TCTGAAAATC

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1751 CAAAATGTTC TATAAAATCA ATGACGCGTT TTAAACCATT AAAAACAGCA
1801 TAGCCACTAT TAAATGGCAT TTTTCTAAAA TACAAATCAA ATACAGCCAT
1851 TTTTTCATGA ATATTATCAT TCCAATAACT TTCAGCCATA TTTATTTGAT
1901 ATAAGTCATT ATGTAACATT AAACTGTCGT CTTCTAATTG GTACACTTGT
1951 ATCTCTCCAA TCGACCTAAA TATTTTCTTA CATTTTATCA TAATTCATTT
2001 TTTTATATAC ATAAGAGCCC CTTAATTTCC ATACTTTTAA TTAAAATCAA
2051 CCAACAATTT AATGACATAT ACATAATTTT TAAGAGTATT TTAATAATGT
2101 AGACTATAAT ATAAAGCGAG GTGTTGTTAA TGTTATTTAA AGAGGCTCAA
2151 GCTTTCATAG AAAACATGTA TAAAGAGTGT CATTATGAAA CGCAAATTAT
2201 CAATAAACGT TTACATGACA TTGAACTAGA AATAAAAGAA ACTGGGACAT
2251 ATACACATAC AGAAGAAGAA CTTATTTATG GTGCTAAAAT GGCTTGGCGT
2301 AATTCAAATC GTTGCATTGG TCGTTTATTT TGGGATTCGT TAAATGTCAT
2351 TGATGCAAGA GATGTTACTG ACGAAGCATC GTTCTTATCA TCAATTACTT
2401 ATCATATTAC ACAGGCTACA AATGAAGGTA AATTAAAGCC GTATATTACT
2451 ATATATGCTC CAAAGGATGG ACCTAAAATT TTCAACAATC AATTAATTCG
2501 CTATGCTGGC TATGACAATT GTGGT
```

Mutant: NT 325

Phenotype: temperature sensitivity

Sequence map: Mutant NT325 is complemented by plasmid pMP644, which carries a 2.1 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 74; no apparent sites for HinD III, EcoR I, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal significant peptide-level similarities to the *ribC* gene product, a protein exhibiting regulatory functions, from *B. subtilis* (Genbank Accession No. x95312; unpublished).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP644, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP644

SEQ ID NO. 85

pMP644 Length: 2181 nt

1 ATCGATAGGA AGAAGTACAA CGACTGAAGA TCAAACGGGT GATACATTGG
51 AAACAAAAGG TGTACACTCA GCAGATTTTA ATAAGGACGA TATTGACCGA
101 TTGTTAGAAA GTTTTAAAGG TATCATTGAA CAAATTCCGC CGATGTACTC
151 ATCCGTCAAA GTAAATGGTA AAAAATTATA TGAATATGCG CGTAATAATG
201 AAACAGTTGA AAGACCAAAG CGTAAAGTTA ATATTAAAGA CATTGGGCGT
251 ATATCTGAAT TAGATTTTAA AGAAAATGAG TGTCATTTTA AAATACGCGT
301 CATCTGTGGT AAAGGTACAT ATATTAGAAC GCTAGCAACT GATATTGGTG
351 TGAAATTAGG CTTTCCGGCA CATATGTCGA AATTAACACG AATCGAGTCT
401 GGTGGATTTG TGTTGAAAGA TAGCCTTACA TTAGAACAAA TAAAAGAACT
451 TCATGAGCAG GATTCATTGC AAAATAAATT GTTTCCTTTA GAATATGGAT
501 TAAAGGGTTT GCCAAGCATT AAAATTAAAG ATTCGCACAT AAAAAAACGT
551 ATTTTAAATG GGCAGAAATT TAATAAAAAT GAATTTGATA ACAAAATTAA
601 AGACCAAATT GTATTTATTG ATGATGATTC AGAAAAAGTA TTAGCAATTT
651 ATATGGTACA CCCTACGAAA AGAATCAGAA ATTAAACCTA AAAAAGTCTT
701 TAATTAAAGG AGATAGAATT TATGAAAGTT CATAGAAAGT GACACATCCT
751 ATACAATCCT AAACAGTTAT ATTACAGGAG GATGTTGCAA TGGGCATTCC
801 GGATTTTTCG ATGGCATGCA TAAAGGTCAT GACAAAGTCT TTGATATATT
851 AAACGAAATA GCTGAGGCAC GCAGTTTAAA AAAAGCGGTG ATGACATTTG
901 ATCCGCATCC GTCTGTCGTG TTTGAATCCT AAAAGAAAAC GAACACGTTT
951 TTACGCCCCT TTCAGATAAA ATCCGAAAAA TTACCCACAT GATATTGATT
1001 ATTGTATAGT GGTTAATTTT TCATCTAGGT TTGCTAAAGT GAGCGTAGAA
1051 GATTTTGTTG AAAATTATAT AATTAAAAAT AATGTAAAAG AAGTCATTGC
1101 TGGTTTTGAT TTTAACTTTT GGTAAATTTG GAAAAGGTAA TATGACTGTA
1151 ACTTCAAGAA TATGATGCGT TTAATACGAC AATTGTGAGT AAACAAGAAA
1201 TTGAAAATGA AAAAATTTCT ACAACTTCTA TTCGTCAAGG ATTTAATCAA
1251 TGGTGAGTTG CCAAAAAGGC GAATGGATGG CTTTTAGGCT ATATATATTT
1301 CTTATTAAAA GGCACTGTAG TGCAAGGTGA AAAAAGGGGA AGAACTATTG
1351 GCTTCCCCAA CAGCTAACAT TCAACCTAGT GATGATTATT TGTTACCTCG
1401 TAAAGGTGTT TATGCTGTTA GTATTGAAAT CGGCACTGAA AATAAATTAT
1451 ATCGAGGGGT AGCTAACATA GGTGTAAAGC CAACATTTCA TGATCCTAAC
1501 AAAGCAGAAG TTGTCATCGA AGTGAATATC TTTGACTTTG AGGATAATAT
1551 TTATGGTGAA CGAGTGACCG TGAATTGGCA TCATTTCTTA CGTCCTGAGA
1601 TTAAATTTGA TGGTATCGAC CCATTAGTTA AACAAATGAA CGATGATAAA
1651 TCGCGTGCTA AATATTTATT AGCAGTTGAT TTTGGTGATG AAGTAGCTTA
1701 TAATATCTAG AGTTGCGTAT AGTTATATAA ACAATCTATA CCACACCTTT
1751 TTTCTTAGTA GGTCGAATCT CCAACGCCTA ACTCGGATTA AGGAGTATTC
1801 AAACATTTTA AGGAGGAAAT TGATTATGGC AATTTCACAA GAACGTAAAA
1851 ACGAAATCAT TAAAGAATAC CGTGTACACG AAACTGATAC TGGTTCACCA
1901 GAAGTACAAA TCGCTGTACT TACTGCAGAA ATCAACGCAG TAAACGAACA
1951 CTTACGTACA CACAAAAAAG ACCACCATTC ACGTCGTGGA TTATTAAAAA
2001 TGGTAGGTCG TCGTAGACAT TTATTAAACT ACTTACGTAG TAAAGATATT
2051 CAACGTTACC GTGAATTAAT TAAATCACTT GGTATCCGTC GTTAATCTTA
2101 ATATAACGTC TTTGAGGTTG GGCATATTTT ATGTTCCAAC CCTTAATTTA
2151 TATTAAAAAA GCTTTTTRCA WRYMTKMASR T

Mutant: NT 333

Ph notype: temperature sensitivity

Sequence map: Mutant NT333 is complemented by plasmid pMP344, which carries a 2.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 75; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal significant similarities to the *murD* gene product from *B. subtilis*, which encodes udp-MurNAc-dipeptide::D-Glu ligase (EC 6.3.2.9); similarities are also noted to the equivalent gene products from *E. coli* and *H. influenzae*. The predicted relative size and orientation of the *murD* gene is depicted by an arrow in the map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP344, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP344

SEQ ID NO. 86
pMP344 Length: 2424 nt

```
  1 ACATTAAAAA GGATGAAATT TGGTCAAAGT ATTCGAGAAG AAGGTCCACA
 51 AAGCCATATG AAGAAGACTG GTACACCAAC GATGGGTGGA CTAACATTTC
101 TATTAAGTAT TGTGATAACG TCTTTGGTGG CTATTATATT TGTAGATCAA
151 GCWAATCCAA TCATACTGTT ATTATTTGTG ACGATTGGTT TTGGGTTAAT
201 TGGTTCTTAT ACGATGATTA TATTATTGTT GTTAAAAAGA ATAACCAAGG
251 TTTAACAAGT AAACAGAAGT TTTTGGCGCA AATTGGTATT GCGATTATAT
301 TCTTTGTTTT AAGTAATGTG TTTCATTTGG TGAATTTTTC TACGAGCATA
351 CATATTCCAT TTACGAATGT AGCAATCCCA CTATCATTTG CATATGTTAT
401 TTTCATTGTT TTTTGGCAAG TAGGTTTTTC TAATGCAGTA AATTTAACAG
451 ATGGTTTAGA TGGATTAGCA ACTGGACTGT CAATTATCGG ATTTACAATG
501 TATGCCATCA TGAGCTTTGT GTTAGGAGAA ACGGCAATTG GTATTTTCTG
551 TATCATTATG TTGTTTGCAC TTTTAGGATT TTTACCATAT AACATTAACC
601 CTGCTAAAGT GTTTATGGGA GATACAGGTA GCTTAGCTTT AGGTGGTATA
651 TTTGCTACCA TTTCAATCAT GCTTAATCAG GAATTATCAT TAATTTTTAT
701 AGGTTTAGTA TTCGTAATTG AAACATTATC TGTTATGTTA CAAGTCGCTA
751 GCTTTAAATT GACTGGAAAG CGTATATTTA AAATGAGTCC GATTCATCAT
801 CATTTTGAAT TGATAGGATG GAGCGAATGG AAAGTAGTTA CAGTATTTTG
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 851  GGCTGTTGGT CTGATTTCAG GTTTAATCGG TTTATGGATT GGAGTTGCAT
 901  TAAGATGCTT AATTATACAG GGTTAGAAAA TAAAAATGTW TTAGTTGTCG
 951  GTTTGGCAAA AAGTGGTTAT GAAGCAGCTA AATTATTAAG TAAATTAGGT
1001  GCGAATGTAA CTGTCAATGA TGGAAAAGAC TTATCACAAG ATGCTCATGC
1051  AAAAGATTTA GAWTCTATGG GCATTTCTGT TGTAAGTGGA AGTCATCCAT
1101  TAACGTTGCT TGATAATAAT CCAATAATTG TTAAAAATCC TGGAATACCC
1151  TTATACAGTA TCTATTATTG ATGAAGCAGT GAAACGAGGT TTGAAAATTT
1201  TAACAGAAGT TGAGTTAAGT TATCTAATCT CTGAAGCACC AATCATAGCT
1251  GTAACGGGTA CAAATGGTAA AACGACAGTT ACTTCTCTAA TTGGAGATAT
1301  GTTTAAAAAA AGTCGCTTAA CTGGAAGATT ATCCGGCAAT ATTGGTTATG
1351  TTTGCATCTA AAGTWGCACA AGAAGTWAAG CCTACAGATT ATTTAGTTAC
1401  AGAGTTGTCG TCATTCCAGT TACTTGGAAT CGAAAAGTAT AAACCACACA
1451  TTGCTATAAT TACTAACATT TATTCGGCGC ATCTAGATTA CCATGRAAAT
1501  TTAGAAAACT ATCAAAATGC TAAAAAGCAA ATATATAAAA ATCAAACGGA
1551  AGAGGATTAT TTGATTTGTA ATTATCATCA AAGACAAGTG ATAGAGTCGG
1601  AAGAATTAAA AGCTAAGACA TTGTATTTCT CAAACTCAAC AAGAAGTTGA
1651  TGGTATTTAT ATTAAAGATG RTTTTATCGT TTATAAAGGT GTTCGTATTA
1701  TTAACACTGA AGATCTAGTA TTGCCTGGTG AACATAATTT AGAAAATATA
1751  TTAGCCAGCT GKGCTKGCTT GTATTTWAGY TGGTGTACCT ATTAAAGCAA
1801  TTATTGATAG TTWAAYWACA TTTTCAGGAA TAGAGCATAG ATTGCAATAT
1851  GTTGGTACTA ATAGAACTTA ATAAATATTA TAATGATTCC AAAGCAACAA
1901  ACACGCTAGC AACACAGTTT GCCTTAAATT CATTTAATCA ACCAATCATT
1951  TGGTTATGTG GTGGTTTGGA TCGGAGGGAA TGAATTTGAC GAACTCATTC
2001  CTTATATGGA AAATGTTCGC GCGATGGTTG TATTCGGACA AACGAAAGCT
2051  AAGTTTGCTA AACTAGGTAA TAGTCAAGGG AAATCGGTCA TTGAAGCGAA
2101  CAATGTCGAA GACGCTGTTG ATAAAGTACA AGATATTATA GAACCAAATG
2151  ATGTTGTATT ATTGTCACCT GCTTGTGCGA GTTGGGATCA ATATAGTACT
2201  TTTGAAGAGC GTGGAGAGAA ATTTATTGAA AGATTCCGTG CCCATTTACC
2251  ATCTTATTAA AGGGTGTGAG TATTGATGGA TGATAAAACG AAGAACGATC
2301  AACAAGAATC AAATGAAGAT AAAGATGAAT TAGAATTATT TACGAGGAAT
2351  ACATCTAAGA AAAGACGGCA AAGAAAAAGW TCCTCTAGAG TCGACCCTGC
2401  AGGCATGCAA GCTTGGCGTA NCC
```

Mutant: **NT 346**

Phenotype: temperature sensitivity

Sequence map: Mutant NT346 is complemented by plasmid pMP347, which carries a 2.1 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 76; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to the *tpiS* gene from *B. subtilis*, which encodes triose phosphate isomerase (EC 5.3.1.1); similarities are also noted to the equivalent gene products from *B. megaterium*

and *B. stearothermophilus*. The predicted relative size and orientation of the *tpiS* gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP347, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP347

SEQ ID NO. 87
pMP347 Length: 2094 nt

```
   1 CACATAAACC AGTTGTTGCT ATTTTAGGTG GAGCAAAAGT ATCTGACAAA
  51 ATTAATGTCA TCAAAAACTT AGTTAACATA GCTGATAAAA TTATCATCGG
 101 CGGAGGTATG GCTTATACTT TCTTAAAAGC GCAAGGTAAA GAAATTGGTA
 151 TTTCATTATT AGAAGAAGAT AAAATCGACT TCGCAAAAGA TTTATTAGAA
 201 AAACATGGTG ATAAAATTGT ATTACCAGTA GACACTAAAG TTGCTAAAGA
 251 ATTTTCTAAT GATGCCAAAA TCACTGTAGT ACCATCTGAT TCAATTCCAG
 301 CAGACCAAGA AGGTATGGAT ATTGGACCAA ACACTGTAAA ATTATTTGCA
 351 GATGAATTAG AAGGTGCGCA CACTGTTGTT ATGGAATGGA CCTATGGGTT
 401 GTTATTCGAG TTCAGTAACT TTGCACAAGG TACAATTGGT GTTTGTTAAA
 451 GCAATTGCCA ACCTTAAAGA TGCCATTACG ATTATCGGTG GCGGTGATTC
 501 AGCCTGCAGC AGCCATCTCT TTAGGTTTTT GAAAATGACT TCACTCMTAT
 551 TTCCACTGGT GGCGGCSCKC CATTAGAKTA CCTAGAAGGT WAAGAATGCC
 601 TGGTWTCMAA GCAAYCAWTA WTAAWTAATA AAGTGATAGT TTAAAGTGAT
 651 GTGGCATGTT TGTTTAACAT TGTTACGGGA AAACAGTCAA CAAGATGAAC
 701 ATCGTGTTTC ATCAACTTTT CAAAAATATT TACAAAAACA AGGAGTTGTC
 751 TTTAATGAGA ACACCAATTA TAGCTGGTAA CTGGAAAATG AACAAAACAG
 801 TACAAGAAGC AAAAGACTTC GTCAATACAT TACCAACACT ACCAGATTCA
 851 AAAGAAKTWR AATCAGTWAT TTGTTGCMCC AGCMATTCAA TTAGATGCAT
 901 TAACTACTGC AGTTWAAGAA GGAAAAGCAC AAGGTTTAGA AATCGGTGCT
 951 CAAAATNCGT ATTTCGAAGA AATGGGGCTT MACAGTGAAA KTTTCCAGTT
1001 GCATAGCAGA TTAGGCTTAA AAAGTTGTAT TCGGTCATTC TGAACTTCGT
1051 GAATATTCCA CGGAACCAGA TGAAGAAATT AACAAAAAAG CGCACGTATT
1101 TTCAAACATG GAATGAMTCC AATTATATGT GTTGGTGAAA CAGACGAAGA
1151 GCGTGAAAGT GGTAAAGCTA ACGATGTTGT AGGTGAGCAA GTTAAAGAAA
1201 GCTGTTGCAG GTTTATCTGA AGATCAAACT TAAATCAGTT GTAATTGCTT
1251 ATGAACCAAT CTGGGCAATC GGAACTGGTA AATCATCAAC ATCTGAAGAT
1301 GCAAATGAAA TGTGTGCATT TGTACGTCAA ACTATTGCTG ACTTATCAAG
1351 CAAAGAAGTA TCAGAAGCAA CTCGTATTCA ATATGGTGGT AGTGTTAAAC
1401 CTAACAACAT TAAAGAATAC ATGGCACAAA CTGATATTGA TGGGGCATTA
1451 GTAGGTGGCG CATCACTTAA AGTTGAAGAT TTCGTACAAT TGTTAGAAGG
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1501  TGCAAAATAA TCATGGCTAA GAAACCAACT GCGTTAATTA TTTTAGATGG
1551  TTTTGCGAAC CGCGAAAGCG AACATGGTAA TGCGGTAAAA TTAGCAAACA
1601  AGCCTAATTT TTNGATCGGT TNATTACCAA CCAAATATCC CAACCGAACT
1651  TCAAAATTCG AAGGCGAGTG GCTTAAGATG TTGGACTACC CTGAAGGACA
1701  AATGGGTAAC TCAGAAGTTG GTCATATGAA TATCGGTGCA GGACGTATCG
1751  TTTATCAAAG TTTAACTCGA ATCAATAAAT CAATTGAAGA CGGTGATTTC
1801  TTTGAAAATG ATGTTTTAAA TAATGCAATT GCACACGTGA ATTCACATGA
1851  TTCAGCGTTA CACATCTTTG GTTTATTGTC TGACGGTGGT GTACACAGTC
1901  ATTACAAACA TTTATTTGCT TTGTTAGAAC TTGCTAAAAA ACAAGGTGTT
1951  GAAAAAGTTT ACGTACACGC ATTTTTAGAT GGCCGTGACG TAGATCAAAA
2001  ATCCGCTTTG AAATACATCG AAGAGACTGA AGCTAAATTC AATGAATTAG
2051  GCATTGGTCA ATTTGCATCT GTGTCTGGTC GTTATTATGC ANTG
```

Mutant: NT348

phenotype: temperature sensitivity

Sequence map: : Mutant NT348 is complemented by plasmid pMP649, which carries a 3.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 77; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal DNA sequence identities to two different Genbank entries for *S. aureus* DNA. The left-most contig below matches Genbank Accession No. U31979, which includes the complete *aroC* gene, encoding 5-enolpyruvylshikimate 3-phosphate phospholyase (EC 4.6.1.4), and the N-terminal portion of the *aroB* gene, encoding 5-dehydroquinate hydrolyase (EC 4.2.1.10); the right-most contig matches Genbank Accession No. L05004, which includes the C-terminal portion of the *aroB* gene. Neither Genbank entry described contains the complete DNA sequence of pMP649. Further experiments are underway to determine whether one or both of the genes identified in clone pMP649 are essential.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP649, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP649

SEQ ID NO. 88

pMP649.forward Length: 954 nt

```
  1 GGGGWYYCTC TAGAGYCGAC CTRCAGGCAT SCAAGCTTBA CCAGGWTCAA
 51 TTAGAGGTRA TTWAGGTTTA RCTKTTSGTV GAADTATCAT BMTCGGTTCA
101 GATTCCTGAG AGTCTGCTGA ACGTGAAATT AATCTATGGT TTAATGAAAA
151 TGAAATTACT AGCTATGCTT CACCACGTGA TGCATGGTTA TATGAATAAA
201 ATATAAACTG TAAACCTTTA CGATTTATTT ATAAAGGTAG AAAGGGTTTT
251 GTTATGTGGT TAGTCATTAT GATTATACAT AACAAGGCCC GTTTTTTATG
301 TTGTAGTAAA TTACTTGAAA AATTTTATAG TTTTTTGGTA ACACGTATTA
351 AAAAGAGAGG AATATTCTTT ATCAAATGAA ACTAAACAGA GAGAAGGGGT
401 TGTTAAAATG AAGAATATTA TTTCGATTAT TTTGGGGATT TTAATGTTCT
451 TAAAATTAAT GGAATTACTA TATGGTGCTA TATTTTTAGA TAAACCACTT
501 AATCCTATAA CAAAAATTAT TTTTATACTG ACTCTCATTT ATATTTTTTA
551 TGTATTAGTA AAAGAATTGA TTATATTTTT GAAGTCAAAG TATAACAAAA
601 GCGCTTAACA TATGTTTATT TTAATATCAT AATTTTTTTA AACGGGACTG
651 ATTAACYTTT ATTAATAATT AACAGTTCGT TCTTTTGTAT TAAGAAATGT
701 AGTCAGTATA TTATTTGCTA AAGTTGCGAT ACGATTATAT TAAAACGGCT
751 AATCATTTTT AATTAATGAT TATATGATGC AACTGTTTAG AAATTCATGA
801 TACTTTTCTA CAGACGAATA TATTATAATT AATTTTAGTT CGTTTAATAT
851 TAAGATAATT CTGACATTTA AAATGAGATG TCATCCATTT TCTTAATTGA
901 GCTTGAAAAC AAACATTTAT GAATGCACAA TGAATATGAT AAGATTAACA
951 ACAT
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SEQ ID NO. 89

pMP649.reverse Length: 841 nt

```
  1 CTTTMAWKRC CTRAACCACT TAACAAACCT GCCAATAATC GTGTTGTCGT
 51 ACCAGAATTA CCTGTATACA ATACTTGATG TGGCGTGTTA AAAGATTGAT
101 ATCCTGGGGA AGTCACAACT AATTTTTCAT CATCTTCTTT GATTTCTACA
151 CCTAACAGTC GGAAAATGTC CATCGTACGA CGACAATCTT CGCCAAGTAG
201 TGGCTTATAT ATAGTAGATA CACCTTCAGC TAGCGACGCC AACATGATTG
251 CACGGTGTGT CATTGACTTA TCGCCCGGCA CTTCTATTTC GCCCTTTAAC
301 GGACCTGAAA TATCAATGAT TTGTTCATTT ACCATTTCAT TCACCTACTT
351 AAAATATGTT TTTAATTGTT CACATGCATG TTGTAATGTT AGTTGATCAA
401 CATGTTGTAC AACGATATCT CCAAATTGTC TAATCAAGAC CATTTGTACA
451 CCTTGCTTAT CATTCTTTTT ATCACTTAGC ATATATTGGT ATAACGTTTC
501 AAAATCCAAG TCAGTTATCA TGTCTAAAGG ATAGCCGAGT TGTATTAAAT
551 ATTGAATATA ATGATTAATA TCATGCTTAG RATCAAACAA AGCATTCGCA
601 ACTATAAATT GATAGATAAT GCCAACCATC ACTGACATGA CCATGAGGTA
651 TTTTATGATA GTATTCAACA GCATGACCAA ATGTATGACC TAAATTTAAR
701 AATTTACGTA CACCTTGTTC TTTTTSATCT GGCGAATAAC AATATCCAGC
751 TTSGTTTCAA TACCTTTRGS AATWTATTTR TCCATACCAT TTAATGACTG
801 TAATATCTCT CTATCTTTAA AGTGCTGTTC GATATCTTGC G
```

Mutant: NT359

phenotype: temperature sensitivity

Sequence map: : Mutant NT359 is complemented by plasmid pMP456, which carries a 3.2 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 78; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal identity to the *glnRA* locus of *S. aureus* (Genbank Accession No. X76490), also referred to as the *femC* locus; mutations localized to *femC* have been reported in the scientific literature to display an increased sensitivity to the bacterial cell-wall synthesis inhibitor methicillin.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP456, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP456

SEQ ID NO. 90
pMP456.forward Length: 568 nt

```
  1 CCGGGGATCC TCTAGAGTCG ATCTTTGCAT TCTTTAAGCT TAAATTTTCT
 51 ATTCTTCTTT CTCTACGGCG CATAGCATTA ATATTACCGT AACTTATCCC
101 AGTATCTTTA TTAATTTGAT AACTCGATAT CTCTTTGTTT TCTATCAATT
151 CTTTGATTGT ATTGAATATT TCATCATAGC AATTCATAAA TTAGATGAGG
201 CGAAATTTTT AATTTTTTAG AATATCAATA GTANTATAAC TAAAATGAAA
251 ATACCGATCG ATAAACAAAA AGATATTTTT TGTTTTGTTT CTCTTTTCAT
301 ATAGTATTAC CCCCTTAATA ATGCGTAGTA AGGTCCCTCT TTTCGGGGTC
351 TTACCTTANA AACGTTCTGC AAATGAATTC GATGAGAAGT AATATGAATA
401 TGGCTATTTT CAAGTAATAC TCAACGTTTT CGCGACGTTC TTTTATCGCC
451 TCATCTCATC ACCTCCAAAT ATATTAAAAT TCATGTGAAC TAAAATATAA
501 AATGGTCTTC CCCAGCTTTA AAAAAATAAA TACATAAAAC ATTTTACTTG
551 GACCAAAACT TGGACCCC
```

SEQ ID NO. 91
pMP456.reverse Length: 581 nt

```
  1 ATGCCTGCAG GTCGATCATT AATTAAAAAC CCTGGCGGTG GTTTAGCTAA
```

51 GATTGGTGGA TACATTGCTG GTAGAAAAGA TTTAATTGAA CGATGTGGTT
101 ATAGATTGAC AGCACCTGGT ATTGGTAAAG AAGCGGGTGC ATCATTAAAT
151 GCATTGCTTG AAATGTATCA AGGTTTCTTT TTAGCACCAC ACGTTGTCAG
201 TCAGAGTCTT AAAGGTGCAT TGTTTACTAG TTTATTTTTA GAAAAAATGA
251 ATATGAACAC AACGCCGAAG TACTACGAAA AACGAACTGA TTTAATTCAA
301 ACAGTTAAAT TTGAAACGAA AGAACAAATG ATTTCATTTT GTCAAAGTAT
351 TCAACACGCA TCCCCAATTA ATGCACATTT TAGTCCANAA CCTAGTTATA
401 TGCCTGGTTA CGAAGATGAT GTTATTATGG CAGCTGGTAC GTTTATTCAA
451 GGTTCATCCG ATTGAATTAT CTGCAGATGG ACCTATTCGT CCTCCTTATG
501 AAGCATATGT TCAAGGANGA TTAACATATG AACACGTTAA AATTGCTGTT
551 GACAAGANCT GTTTAATCAG TTTGAAAAAA C

Mutant: NT371

phenotype: temperature sensitivity

Sequence map: : Mutant NT371 is complemented by plasmid pMP461, which carries a 2.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 79. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *yluD*, a hypothetical ABC transporter (Genbank Accession No. M90761), and *yidA*, a hypothetical ORF of unknown function (Genbank Accession No. L10328).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP461, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP461

SEQ ID NO. 92

pMP461 Length: 2001 nt

1 CGGGGATCCT CTAAAGTCGA TCAAATTGGG CGAATGAAGC AAGGAAAAAC
51 AATTTTAAAA AAGATTTCTT GGCAAATTGC TAAAGGTGAT AAATGGATAT
101 TATATGGGTT GAATGGTGCT GGCAAGACAA CACTTCTAAA TATTTTAAAT
151 GCGTATGAGC CTGCAACATC TGGAACTGTT AACCTTTTCG GTAAAATGCC
201 AGGCAAGGTA GGGTATTCTG CAGAGACTGT ACGACAACAT ATAGGTTTTG

```
 251 TATCTCATAG TTTACTGGAA AAGTTTCAAG AGGGTGAAAG AGTAATCGAT
 301 GTGGTGATAA GCGGTGCCTT TAAATCAATT GGTGTTTATC AAGATATTGA
 351 TGATGAGATA CGTAATGAAG CACATCAATT ACTTAAATTA GTTGGAATGT
 401 CTGCTAAAGC GCAACAATAT ATTGGTTATT TATCTACCGG TGAAAAACAA
 451 CGAGTGATGA TTGCACGAGC TTTAATGGGG CAACCCCAGG TTTTAATTTT
 501 AGATGAGCCA GCAGCTGGTT TAGACTTTAT TGCACGAGAA TCGTTGTTAA
 551 GTATACTTGA CTCATTGTCA GATTCATATC CAACGCTTGC GATGATTTAT
 601 GTGACGCACT TTATTGAAGA AATAACTGCT AACTTTTCCA AAATTTTACT
 651 GCTAAAAGAT GGCCAAAGTA TTCAACAAGG CGCTGTAGAA GACATATTAA
 701 CTTCTGAAAA CATGTCACGA TTTTTCCAGA AAAATGTAGC AGTTCAAAGA
 751 TGGAATAATC GATTTTCTAT GGCAATGTTA GAGTAAATAT TTTGCAAATA
 801 ATAAGTAATA ATGACAAAAT TTAATTAAGA TAAAATGGAC AGTGGAGGGC
 851 AATATGGATA ACGTTAAAAG CAATATTTTT GGACATGGAT GGAACAATTT
 901 TACATTGAAA ATAATCCAAG CATCCAACGT WTACGAAAGA TGTTCATTAA
 951 TCAATTGGAG AGAGAAAGGA TATWAAGTAT TTTTGGSCAA CAGGACGTTC
1001 GCATTCTGAA ATACATCMAA YTTGTACCTC AAGATTTTGC GGTTAATGGC
1051 ATCATTAGTT CAAATGGAAC AATTGGAGAA GTAGATGGAG AAATTATCTT
1101 CAAGCATGGT TTATCATTGG CTCAAGTGCA ACAAATTACT AATTTAGCTA
1151 AGCGCCAACA AATTTATTAT GAGGTATTTC CTTTTGAAGG TAATAGAGTT
1201 TCTTTAAAAG AAGATGAAAC ATGGATGCGA GATATGATTC GTAGTCAAGA
1251 TCCTATTAAT GGCGTAAGTC ATAGTGAATG GTCTTCAAGA CAAGATGCGC
1301 TTGCTGGTAA GATAGATTGG GTAACTAAGT TTCCTGAAGG TGAATATTCA
1351 AAAATTTATC TATTCAGTTC TAATTTAGAA AAAATAACAG CATTTAGAGA
1401 TGAATTAAAG CAAAATCATG TGCAACTACA GATTAGTGTT TCAAATTCAT
1451 CAAGATTTAA TGCGGAAACA ATGGCTTATC AAACTGATAA AGGTACAGGC
1501 ATTAAAGAAA TGATTGCACA TTTTGGTATT CATCAAGAAG AAACGTTAGT
1551 TATTGGAGAT AGCGACAATG ATAGAGCAAT GTTTGAATTT GGTCATTATA
1601 CAGTTGCTAT GAAAAATGCA CGCCCTGAAA TCCAAGCATT AACTTCAGAT
1651 GTAACGGCAT ACACGAATGA AGAGGATGGC GCAGCAAAAT ATTTAGCAGA
1701 GCATTTTTTA GCTGAATAAT AAAATAGGTA GTTATTTATT ATTTAATTTA
1751 CAATAGTTGA TGAGTAATGT ACAAAGAGCA GTAAAGTTAT TTTCTATTAG
1801 AAAATGTCTT ACTGCTCTTT TGTATGCTTA TAAATATTTG AATCATCTAT
1851 ATTTAATTGG ACAAACTCTA TGAGAATAAA TATTGTTAAA ACTAATAAGA
1901 TAGGAAATTC ATTGATTTTG AATAATATTT CTTGTTTTAA GGTTTAACTA
1951 TTGAATTGTA TACTTCTTTT TTTAGTAGCA ACAGATCGAC CTGCAGGCAT
2001 A
```

Mutant: NT 379

Phenotype: temperature sensitivity

Sequence map: Mutant NT379 is complemented by plasmid pMP389, which carries a 2.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 80; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to

the *tagF* gene from *B. subtilis*, which encodes a protein involved in the biosynthesis of teichoic acid polymers (Genbank Accession No. X15200). The Tag genes of *B. subtilis* have been identified as essential and are expected to make good candidates for screen development. The predicted relative size and orientation of the *tagF* gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP389, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP389

SEQ ID NO. 93

pMP389 Length: 2522 nt

```
   1 GANCTCGGTA CCCGGGGATG CCTSYAGAGT CGATCGCTAC CACCTTGAAT
  51 GACTTCAATT CTTTCATCAG AAATTTTGAA TTTTCTAAGT GTATCTTTCG
 101 TATGCGTCAT CCATTGTTGT GGCGTCGCGA TAATAATTTT TTCAAAATCA
 151 TTAATTAAAA TAAATTTTTC TAATGTATGG ATTAAAATCG GTTTGTTGTC
 201 TAAATCTAAA AATTGTTTAG GTAAAGGTAC GTTACCCATT CTTGAGCCTA
 251 TACCTCCAGC TAGAATACCA GCGTATTTCA TAAAATACTT CCTCCATTCA
 301 ACTATATCTA TATTTAATTA TTTAAATTTC GTTGCATTTT CCAATTGAAA
 351 ACTCATTTTA AAATCAAAAC TCTAAATGTC TGTGTATTAC TTAAAATTAT
 401 ACATATTTTG CTTATATTTT AGCATATTTT GTTTAAACCT ATATTACATT
 451 ATATCAGACG TTTTCATACA CAAATAATAA CATACAAGCA AACATTTCGT
 501 TTATTATTTA TATCACTTAA CTAATTAATT TATAATTTTT TATTGTTTTT
 551 AAGTTATCAC TTAAAAATCG TTTGGCAAAT TCGTTGTGAC GCTTGTCCAT
 601 CTTCTAATGA ACAGAATTTT TGATAAAATA CCGTTCGTGC TTCAATATAC
 651 TCATTTGCAG TCTCATCGAT TTGTTTTAAT GCATCAATGA GTGCTGTTTG
 701 ATTTTCAACA ATTGGAMCTG GCAACTCTTT TTTATAATCC ATGTAAAAAC
 751 CTCTAAGCTC ATCGCCATAT TTATCTAAGT CATATGCATA GAAAATTTGC
 801 GGACGCTTTA ATACACCGAA GTCGAACATG ACAGATGAGT AGTCGGTAAC
 851 TAACGCATCG CTGATTAAGT TATAAATCCG AAATGCCTTC ATAATCTGGA
 901 AAMGTCTTTC AACAAAATCA TCAATGTTCA TCAATAACGY GTCAACAACT
 951 AAATAATGCA KGCGTAATAA AATAACATAA TCATCATCCA GCGCTTGACG
1001 CAAAGCTTCT ATATCAAAGT TAACATTAAA TTGATATGAA CCCTTCTCGG
1051 AATCGCTTCA TCGTCAACGC CAAGTTGGCG CGTACATAAT CAACTTTTTT
1101 ATCTAATGGA ATATTTAATC TTGTCTTAAT ACCATTAATA TATTCAGTAT
1151 CATTGCGTTT ATGTGATAAT TTATCATTTC TTGGATAACC TGTTTCCAAA
1201 ATCTTATCTC GACTAACATG AAATGCATTT TGAAATATCG ATGTCGAATA
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1251  TGGATTAGGT GACACTAGAT AATCCCACCG TTGGCTTTCT TTTTTAAAGC
1301  CATCTTGGTA ATTTTGAGTA TTTGTTCCTA GCATTTTAAC GTTACTAATA
1351  TCCAAACCAA TCTTTTTTAA TGGCGTGCCA TGCCATGTTT GTAAGTACGT
1401  CGTTCGCGGT GATTTATATA ACCAATCTGG TGTACGTGTG TTAATCATCC
1451  ACGCTTTCGC TCTTGGCATC GCTAAAAACC ATTTCATTGA AAACTTTGTA
1501  ACATATGGTA CATTGTGCTG TTGGAATATG TGTTCATATC CTTTTTTCAC
1551  ACCCCATATT AATTGGGCAT CGCTATGTTC AGTTAAGTAT TCATATAATG
1601  CTTTGGGGTT GTCGCTGTAT TGTTTACCAT GAAAGCTTTC AAAATAAATT
1651  AGATTCTTGT TTGGCAATTT TGGATAGTAA TTTAAAAGTC GTATATATAC
1701  TATGTTCTAT CAATTTTTTA ATTGTATTTT TAATCATGTC GTACCTCCGA
1751  CGTGTTTTTG TAATTATATT AATATGTATG AGCAAGCTCA TTGTAACCAT
1801  GCCTATTATA GCATTTCATC ATAAAATACA TTTAACCATT ACACTTGTCG
1851  TTAATTATCA TACGAAATAC ATGATTAATG TACCACTTTA ACATAACAAA
1901  AAATCGTTAT CCATTCATAA CGTATGTGTT TACACATTTA TGAATTAGAT
1951  AACGATTGGA TCGATTATTT TATTTWACAA AATGACAATT CAGTTGGAAG
2001  GTGATTGCTT TTGATTGAAT CGCCTTATGC ATGAAAAATC AAAAGGTTAT
2051  TCTCATTGTA TAGTCCTGCT TCTCATCATG ACATGTTGCT CACTTCATTG
2101  TCAGAACCCT TCTTGAAAAC TATGCCTTAT GACTCATTTG CATGGCAAGT
2151  AATATATGCC AACATTAGCG TCTAAACAAA TCTTTGACTA AACGTTCACT
2201  TGAGCGACCA TCTTGATATT TAAAATGTTT ATCTAAGAAT GGCACAACTT
2251  TTTCAACCTC ATAATCTTCA TTGTCCAAAG CATCCATTAA TGCATCAAAG
2301  GACTGTACAA TTTTACCTGG AACAAATGAT TCAAATGGTT CATAGAAATC
2351  ACGCGTCGTA ATGTAATCTT CTAAGTCAAA TGCATAGAAA ATCATCGGCT
2401  TTTTAAATAC TGCATATTCA TATATTAAAG ATGAATAATC ACTAATCAAC
2451  AAGTCTGTAA CAAAGAGAAT ATCGTTWACT TCASGRTCGA TCGACTCTAG
2501  AGGATCCCCG GGTACCGAGC TC
```

Mutant: NT 380

Phenotype: temperature sensitivity

Sequence map: Mutant NT380 is complemented by plasmid pMP394, which carries a 1.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 81. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to the *cdsA* gene product from *E. coli* (Genbank Accession No. M11330), which encodes phosphatidate cytidylyltransferase (EC 2.7.7.41); the *cdsA* gene product is involved in membrane biogenesis and is likely to be a good candidate for screen development. The predicted relative size and orientation of the *cdsA* gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP394, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP394
SEQ ID NO. 94

pMP394 Length: 1335 nt

```
   1 CAGAGTTGTT AATTCGTACT TCAGGAGAAC AAAGAATAAG TAATTTCTTG
  51 ATTTGGCAAG TTTCGTATAG TGAATTTATC TTTAATCAAA AATTATGGCC
 101 TGACTTTGAC GAAGATGAAT TAATTAAATG TATAAAAATT TATCAGTCAC
 151 GTCAAAGACG CTTTGGCGGA TTGARTGAKG AGKATRTATA GTATGAAAGT
 201 TAGAACGCTG ACAGCTATTA TTGCCTTAAT CGTATTCTTG CCTATCTTGT
 251 TAAAAGGCGG CCTTGTGTTA ATGATATTTG CTAATATATT AGCATTGATT
 301 GCATTAAAAG AAATTGTTGA ATATGAATAT GATTAAATTT GTTTCAGTTC
 351 CTGGTTTAAT TAGTGCAGTT GGTCTTATCA TCATTATGTT GCCACAACAT
 401 GCAGGGCCAT GGGTACAAGT AATTCAATTA AAAAGTTTAA TTGCAATGAG
 451 CTTTATTGTA TTAAGTTATA CTGTCTTATC TAAAAACAGA TTTAGTTTTA
 501 TGGATGCTGC ATTTTGCTTA ATGTCTGTGG CTTATGTAGG CATTGGTTTT
 551 ATGTTCTTTT ATGAAACGAG ATCAGAAGGA TTACATTACA TATTATATGC
 601 CTTTTTAATT GTTTGGCTTA CAGATACAGG GGCTTACTTG TTTGGTAAAA
 651 TGATGGGTTA AACATAAGCT TTGGCCAGTA ATAAKTCCGA ATAAAACAAT
 701 CCGAAGGATY CATAGGTGGC TTGTTCTGTA GTTTGATAGT ACCACTTGCA
 751 ATGTTATATT TTGTAGATTT CAATATGAAT GTATGGATAT TACTTGGAGT
 801 GACATTGATT TTAAGTTTAT TTGGTCAATT AGGTGATTTA GTGGAATCAG
 851 GATTTAAGCG TCATTTNGGC GTTAAAGACT CAGGTCGAAT ACTACCTGGA
 901 CACGGTGGTA TTTTAGACCG ATTTGACAGC TTTATGTTTG TGTTACCATT
 951 ATTAAATATT TTATTAATAC AATCTTAATG CTGAGAACAA ATCAATAAAC
1001 GTAAAGAGGA GTTGCTGAGA TAATTTAATG AATCCTCAGA ACTCCCTTTT
1051 GAAAATTATA CGCAATATTA ACTTTGAAAA TTATACGCAA TATTAACTTT
1101 GAAAATTAGA CGTTATATTT TGTGATTTGT CAGTATCATA TTATAATGAC
1151 TTATGTTACG TATACAGCAA TCATTTTTAA AATAAAAGAA ATTTATAAAC
1201 AATCGAGGTG TAGCGAGTGA GCTATTTAGT TACAATAATT GCATTTATTA
1251 TTGTTTTTGG TGTACTAGTA ACTGTTCATG AATATGGCCA TATGTTTTTT
1301 GCGAAAAGAG CAGGCATTAT GTGTCCAGAA TTTGC
```

Mutant: NT401
phenotype: temperature sensitivity

Sequence map: Mutant NT401 is complemented by plasmid pMP476, which carries a 2.9 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 82. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal sequence identity in the middle of the clone to pMP64, the complementing clone to NT31 (described previously). Since pMP64 does not cross complement NT401, and pMP476 contains additional DNA both upstream and downstream, the essential gene is likely to reside in the flanking DNA. The remaining DNA that completely contains an ORF is that coding for *yqeJ*, a hypothetical ORF from *B. subtilis* (Genbank Accession No. D84432)

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP476, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP476

SEQ ID NO. 95
pMP476 Length: 2902 nt

```
  1  GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GATCATTACC TAATTCGTAT
 51  TGTCGAACAA TTTGATACAT TTTACCTAAA TCATCATATT TACAGAAATC
101  ATGTAATACA CCTGCTAATT CTACTTTACT AGTGTCTCCA TCATAAATTT
151  CTGCCRATTT AATCGCTGTT TCTGCAACTC TTAAAGAATG ATTGATRACG
201  TTTCTCTGGA CAGTTTCTCT TTTGCAAGCC GTTTTGCTTT TTCAATGTWC
251  ATATAATCCT TCCCCCTTAA TATAGTTTTC AACGGATTTA GGAACAAGAA
301  CTTGGATAGA TTTCCCTTCA CTAACTCTTT GTCGAATCAT TGTCGAACTT
351  ATATCTACCC TAGGTATCTG AATTGCAATC ATAGCATTTT CAACATTTTG
401  ACTATTTTTG TCTCGATTTA CAACTACAAA AGTAACCATT TCTTTTAAGT
451  ATTCAATTTG ATACCATTTC TCTAGTTGGT TATACTGATC CGTCCCAATA
501  ACAAAGTACA ACTCACTGTC TTTGTGTTGC TCCTTGAATG CCTTGATCGT
551  GTCATAGGTA TAACTTTGAC CACCACGTTT AATTTCATCG TCACAAATAT
601  CTCCAAAACC AAGCTCGTCG ATAATCATCT GTATCATTGT TAATCTGTGC
651  TGAACGTCTA TAAAATCATG GTGCTTTTTC AATGGAGAMA WAAAAMWARR
701  WAAAAAATAA AATTCATCTG GCTGTAATTC ATGAAATACT TCGCTAGCTA
751  CTATCATATG TTGCAGTATG GATAGGGTTA AACTGACCGC CGTAAAGTAC
801  TATCTTTTTC ATTATTATGG CAATTCAATT TCTTTATTAT CTTTAGATTC
851  TCTATAAATC ACTATCATAG ATCCAATCAC TTGCACTAAT TCACTATGAA
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901 KTAGCTTCCG CTTAATGTTT CCAGCTAATY CTTTTTTATC ATCAAAGTTT
951 ATTTTGTTAK TACATGTTAC TTTAATCAAT YCTCTGTTTT CYAACGTTAT
1001 CATCTATTTG TTTAATCATA TTTTCGTTGA TACCGCCTTT TCCAATTTGA
1051 AAAATCGGAT CAATATTGTG TGCTAAACTT CTTAAGTATC TTTTTTGTTT
1101 GCCAGTAAGC ATATGTTATT CTCCTTTTAA TTGTTGTAAA ACTGCTGTTT
1151 TCATAGAATT AATATCAGCA TCTTTATTAG TCCAAATTTT AAAGCTTTCC
1201 GCACCCTGGT AAACAAACAT ATCTAAGCCA TTATAAATAT GGTTTCCCTT
1251 GCGCTCTGCT TCCTCTAAAA TAGGTGTTTT ATACGGTATA TAAACAATAT
1301 CACTCATTAA AGTATTGGGA GAAAGAGCTT TAAATTAATA ATACTTTCGT
1351 TATTTCCAGC CATACCCGCT GGTGTTGTAT TAATAACGAT ATCGAATTCA
1401 GCTAAATACT TTTCAGCATC TGCTAATGAA ATTTGGTTTA TATTTAAATT
1451 CCAAGATTCA AAACGAGCCA TCGTTCTATT CGCAACAGTT AATTTGGGCT
1501 TTACAAATTT TGCTAATTCA TAAGCAATAC CTTTACTTGC ACCACCTGCG
1551 CCCAAAATTA AAATGTATGC ATTTTCTAAA TCTGGATAAA CGCTGTGCAA
1601 TCCTTTAACA TAACCAATAC CATCTGTATT ATACCCTATC CACTTGCCAT
1651 CTTTTATCAA AACAGTGTTA ACTGCACCTG CATTAATCGC TTGTTCATCA
1701 ACATAATCTA AATACGGTAT GATACGTTCT TTATGAGGAA TTGTGATATT
1751 AAASCCTTCT AATTYTTTTT TSGAAATAAT TTCTTTAATT AAATGAAAAA
1801 TTYTTCAATT GGGAATATTT AAAGCTTCAT AAGTATCATC TTAATCCTAA
1851 AGAATTAAAA TTTGCTCTAT GCATAACGGG CGACAAGGAA TGTGAAATAG
1901 GATTTCCTAT AACTGCAAAT TTCATTTTTT TAATCACCTT ATAAAATAGA
1951 ATTYTTTAAT ACAACATCAA CATTTTTAGG AACACGAACG ATTACTTTAG
2001 CCCCTGGTCC TATAGTTATA AAGCCTAGAC CAGAGATCAT AACATCGCGT
2051 TTCTCTTTGC CTGTTTCAAG TCTAACAGCC TTTACCTCAT TAAGATCAAA
2101 ATTTTGTGGA TTTCCAGGTG GCGTTAATAA ATCGCCAAGT TGATTACGCC
2151 ATAAATCATT AGCCTTCTCC GTTTTAGTAC GATGTATATT CAAGTCATTA
2201 GAAAAGAAAC AAACTAACGG ACGTTTACCA CCTGAWACAT AATCTATGCG
2251 CGCTAGACCG CCGAAGAATA ATGTCKGCGC CTCATTTAAT TGATATACGC
2301 GTTGTTTTAT TTCTTTCTTA GGCATAATAA TTTTCAATYC TTTTTCACTA
2351 ACTAAATGCG TCATTTGGTG ATCTTGAATA ATACCTGGTG TATCATACAT
2401 AAATGATGTT TCATCTAAAG GAATATCTAT CATATCTAAA GTTGYTTCCA
2451 GGGAATCTTG AAGTTGTTAC TACATCTTTT TCACCAACAC TAGCTTCAAT
2501 CAGTTTATTA ATCAATGTAG ATTTCCCAAC ATTCGTTGTC CCTACAATAT
2551 ACACATCTTC ATTTTCTCGA ATATTCGCAA TTGATGATAA TAAGTCGTCT
2601 ATGCCCCAGC CTTTTTCAGC TGAAATTAAT ACGACATCGT CAGCTTCCAA
2651 ACCATATTTT CTTGCTGTTC GTTTTAACCA TTCTTTAACT CGACGTTTAT
2701 TAATTTGTTT CGGCAATAAA TCCAATTTAT TTGCTGCTAA AATGATTTTT
2751 TTGTTTCCGA CAATACGTTT AACTGCATTA ATAAATGATC CTTCAAAGTC
2801 AAATACATCC ACGACATTGA CGACAATACC CTTTTTATCC GCAAGTCCTG
2851 ATAATAATTT TAAAAAGTCT TCACTTTCTA ATCCTACATC TTGAACTTCG
2901 TT

Mutant: NT423

phenotype: temperature sensitivity

Sequence map: : Mutant NT423 is complemented by plasmid pMP499, which carries a 2.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted

in Fig. 83. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *yqhY*, a hypothetical ORF identified from a genomic sequencing effort in *B. subtilis* (Genbank Accession No. D84432), and *yqhZ*, a hypothetical ORF from *B.* subtilis bearing similarity to the *nusB* gene product from *E. coli* (Genbank Accession No. M26839; published in Imamoto, F. *et al. Adv. Biophys.* **21** (1986) 175-192). Since the *nusB* gene product has been demonstrated to be involved in the regulation of transcription termination in *E. coli*, it is likely that either one or both of the putative genes identified in this sequence contig encode essential functions.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP499, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP499

SEQ ID NO. 96
pMP499 Length: 1916 nt

```
  1 AGTCGATCAA AGCCAATGTT CCAGTTGTTC CTGGTAGTGA CGGTTTAATG
 51 AAAGACGTCT CAGAAGCTAA GAAAATCGCC AAAAAAATTG GCTATCCGGT
101 CATCATTAAA GCTACTGCTG GCGGTGGCGG AAAAGGTATC CGTGTTGCTC
151 GTGATGAAAA AGAACTTGAA ACTGGCTTCC GAATGACAGA ACAAGAAGCT
201 CAAACTGCAT TTGGTAATGG TGGACTTTAT ATGGAGAAAT TCATCGAAAA
251 CTTCCGCCAT ATTGAAATCC AAATTGTTGG GGACAGCTAT GGTAATGTAA
301 TTCATTTAGG AGAACGTGAT TGTACAATTC AAAGACGTNT GCAGAAATTA
351 GTGGAAGAAG CACCTTCCCC NATTTTAGAT GATGAAACAC GTCGTGAAAT
401 GGGAAATGCC GCAGTTCGTG CAGCGAAAGC TGTAAATTAT GAAAATGCGG
451 GAACAATTGA GTTTATATAT GATTTAAATG ATAATAAATT TTATTTTATG
501 GAAATGAATA CACGTATTCA AGTAGAACAT CCTGTAACTG AAATGGTAAC
551 AGGAATTGAT TTAGTTAAAT TACAATTACA AGTTGCTATG GGTGACGTGT
601 TACCGTATAA ACAAGAAGAT ATTAAATTAA CAGGACACGC AATTGAATTT
651 AGAATTAATG CTGAAAATCC TTACAAGAAC TTTATGCCAT CACCAGGTAA
701 AATTGAGCAA TATCTTGCAC CAGGTGGATA TGGTGTTCGA ATAGAGTCAG
751 CATGTTATAC TAATTATACG ATACCGCCAT ATTATGATTC GATGGTAGCG
801 AAATTAATCA TACATGAACC GACACGAGAT GARGCGATTA TGGSTGGCAT
851 TCGTGCACTA ARKGRAWTTG TGGTTYTTGG GTATTGATAC AACTATTCCA
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 901  TTTCCATATT AAATTATTGA ATAACGGATA TATTTAGGAA GCGGTAAATT
 951  TAATACAAAC TTTTTAGAAG CAAAATAGCA TTATTGAATG ATGAAAGGTT
1001  AATAGGAGGT CMATCCCMTG GTCAAAGTAA CTGATTATTC MAATTCMAAA
1051  TTAGGTAAAG TAGAAATAGC GCCAGAAGTG CTATCTGTTA TTGCAAGTAT
1101  AGCTACTTCG GAAGTCGAAG GCATCACTGG CCATTTTGCT GAATTAAAAG
1151  AAACAAATTT AGAAAAAGTT AGTCGTAAAA ATTTAAGCCG TGATTTAAAA
1201  ATCGAGAGTA AAGAAGATGG CATATATATA GATGTATATT GTGCATTAAA
1251  ACATGGTGTT AATATTTCAA AAACTGCAAA CAAAATTCAA ACGTCAATTT
1301  TTAATTCAAT TTCTAATATG ACAGCGATAG AACCTAAGCA AATTAATATT
1351  CACATTACAC AAATCGTTAT TGAAAAGTAA TGTCATACCT AATTCAGTAA
1401  TTAAATAAAG AAAAATACAA ACGTTTGAAG GAGTTAAAAA TGAGTCGTAA
1451  AGAATCCCGA GTGCAAGCTT TTCAAACTTT ATTTCAATTA GAAATGAAGG
1501  ACAGTGATTT AACGATAAAT GAAGCGATAA GCTTTATTAA AGACGATAAT
1551  CCAGATTTAG ACTTCGAATT TATTCATTGG CTAGTTTCTG GCGTTAAAGA
1601  TCACGAACCT GTATTAGACG AGACAATTAG TCCTTATTTA AAAGATTGGA
1651  CTATTGCACG TTTATTAAAA ACGGATCGTA TTATTTTAAG AATGGCAACA
1701  TATGAAATAT TACACAGTGA TACACCTGCT AAAGTCGTAA TGAATGAAGC
1751  AGTTGAATTA ACAAAACAAT TCAGTGATGA TGATCATTAT AAATTTATAA
1801  ATGGTGTATT GAGTAATATA AAAAAATAAA ATTGAGTGAT GTTATATGTC
1851  AGATTATTTA AGTGTTTCAG CTTTAACGAA ATATATTAAA TATAAATTTG
1901  ATCGACCTGC AGGCAT
```

Mutant: NT432

phenotype: temperature sensitivity

Sequence map: : Mutant NT432 is complemented by plasmid pMP500, which carries a 1.9 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 84. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to the *pgsA* gene product, encoding CDP-diacylglycerol:glycerol-3-phosphate 3-phosphatidyltransferase (PGP synthase; EC 2.7.8.5) from *B. subtilis*(Genbank Accession No. D50064; published in Kontinen, V.P. *et al. FEBS lett.* **364** (1995) 157-160).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP500, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of

amplification from genomic DNA with subsequent DNA sequencing.

clone pMP500

SEQ ID NO. 97

pMP500 Length: 1932 nt

```
   1 CGGGGATCCT CTAGAGTCGA TCCGTTTGGT GGTGGTTTTG GTTTCTTCGA
  51 GTAAGTGTAA GGAGGCTATG AATTGARRAC GGTCGGTGAA GCGCTAAAAG
 101 GTANACGTGA AAGGTTAGGA ATGACTTYAA CAGAATTAGA GCAACGTACT
 151 GGAATTAANC GTGAAATGCT AGTGCATATT GAAAATAATG AATTCGATCA
 201 ACTACCGAAT AAAAATTACA GCGAAGGATT TATTAGAAAA TATGCAAGCG
 251 TAGTAAATAT TGAACCTAAC CAATTAATTC AAGCTCATCA AGATGAAATT
 301 CCATCGAACC AGAGCCGAAT GGGACGAAGT AATTACAGTT TTCAATAGAT
 351 AATAAAGACT TACGATTATA AGAGTAAATC AAAGANAGCC AATACAATTA
 401 TTAGTAATCA TGGGTTATTA CAGTTTTAAT AACTTTATTG TTATGGATCA
 451 TGTTAGTTTT AATATTTTAA CAGAAATAAA TTAGTGAGAA ATGAGGATGT
 501 TATAATGAAT ATTCCGAACC AGATTACGGT TTTTAGAGTT AGTGTTAATA
 551 CCAGTTTTTA TATTGTTTGC GTTAGTTGAT TTTGGATTTG GCAATGTGTC
 601 ATTTCTAGGA GGATATGAAA TAAGAATTGA GTTATTAATC AGTGGTTTTA
 651 TTTTTATATT GGCTTCCCTT AGCGATTTTG TTGATGGTTA TTTAGCTAGA
 701 AAATGGAATT TAGTTACAAA TATGGGGAAA TTTTTGGATC CATTAGCGGA
 751 TAAATTATTA GTTGCAAGTG CTTTAATTGT ACTTGTGCAA CTAGGACTAA
 801 CAAATTCTGT AGTAGCAATC ATTATTATTG CCAGAGAATT TGCCGTAACT
 851 GGTTTACGTT TACTACAAAT TGAACAAGGA TTCCGTAAGT TGCAGCTGGT
 901 CCAATTTAGG TWAAAWTWAA AACAGCCAGT TACTATGGTT AGCMAWTWAC
 951 TTGGTTGTTW ATTAAGKTGA TCCCATTGGG CAACATTGAT TGGTTTGTCC
1001 ATTARGACAA ATTTTAATTA TAACATTGGC GTTATWTTTW ACTATCYTAT
1051 CTGGTATTGA ATAACTTTTA TAAAGGTAGA GATGTTTTTA AACAAAAATA
1101 AATATTTGTT TATACTAGAT TTCATTTTCA TATGGAATCT AGTTTTTTTA
1151 ATCCCAATTT TAGAAATTAG CCACGCAATT GTTTATAATG ATATATTGTA
1201 AAACAATATT TGTTCATTTT TTTAGGGAAA ATCTGTAGTA GCATCTGATA
1251 CATTGAATCT AAAATTGATG TGAATTTTTA AATGAAATAC ATGAAAAAAT
1301 GAATTAAACG ATACAAGGGG GATATAAATG TCAATTGCCA TTATTGCTGT
1351 AGGCTCAGAA CTATTGCTAG GTCAAATCGC TAATACCAAC GGACAATTTC
1401 TATCTAAAGT ATTTAATGAA ATTGGACAAA ATGTATTAGA ACATAAAGTT
1451 ATTGGAGATA ATAAAAAACG TTTAGAATCA AGTGTAACGT CATGCGCTAG
1501 AAAAATATGA TACTGTTATT TTAACAGGTG GCTTAGGTCC TACGAAAGAT
1551 GACTTAACGA AGCATACAGT GGCCCAGATT GTTGGTAAAG ATTTAGTTAT
1601 TGATGAGCCT TCTTTAAAAT ATATTGAAAG CTATTTTGAG GAACAAGGAC
1651 AAGAAATGAC ACCTAATAAT AAACAACAGG CTTTAGTAAT TGAAGGTTCA
1701 ACTGTATTAA CAAATCATCA TGGCATGGCT CCAGGAATGA TGGTGAATTT
1751 TGAAAACAAA CAAATTATTT TATTACCAGG TCCACCGAAA GAAATGCAAC
1801 CAATGGTGAA AAATGAATTG TTGTCACATT TTATAAACCA TAATCGAATT
1851 ATACATTCTG AACTATTAAG ATTTGCGGGA ATAGGTGAAT CTAAAGTAGA
1901 AACAATATTA ATAGATCGAC CTGCAGGCAT GC
```

Mutant: NT435

phenotype: temperature sensitivity

Sequence map: Mutant NT435 is complemented by plasmid pMP506, which carries a 3.2 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 85. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarity from the left-most contig (shown below) to the *pdhA* gene product, encoding the E1-alpha subunit of pyruvate dehydrogenase, from *B. subtilis*. The right-most contig below demonstrates DNA sequence identity to the *pdhC* gene, encoding the E2 chain of dihydrolipoamide acetyltransferase (EC 2.3.1.12), from *S. aureus* (Genbank Accession No. X58434). This Genbank entry also contains the *pdhB* gene upstream, encoding the E1-beta subunit of pyruvate dehydrogenase (EC 1.2.4.1); since the pMP506 clone contains the region upstream of *pdhC*, it is predicted that the essential gene identified by mutant NT435 is *pdhB*. Further sequencing is currently underway to prove this assertion.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP506, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP506

SEQ ID NO. 98

pMP506.forward Length: 619 nt

```
  1  ATTCGAGCTC GGTACCCGGG GATCCTCTAN AGTCGATCTT ACGGATGAAC
 51  AATTAGTGGA ATTAATGGAA AGAATGGTAT GGACTCGTAT CCTTGATCAA
101  CGTTCTATCT CATTAAACAG ACAAGGACGT TTAGGTTTCT ATGCACCAAC
151  TGCTGGTCAA GAAGCATCAC AATTAGCGTC ACAATACGCT TTAGAAAAAG
201  AAGATTACAT TTTACCGGGA TACAGAGATG NTCCTCAAAT TATTTGGCAT
251  GGTTTACCAT TAACTGAAGC TTTCTTATTC TCAAGAGGTC ACTTCAAAGG
301  AAATCAATTC CCTGAAGGCG TTAATGCATT AAGCCCACAA ATTATTATCG
351  GTGCACAATA CATTCAAGCT GCTGGTGTTT GCATTTGCAC TTAAAAAACG
401  TTGGTAAAAA TGCAGTTGCA ATCACTTACA CTGGTTGACG GTGGTTCTTC
451  ACAAGGTTGA TTTCTACGAA GGTATTAACT TTGCAGCCAG CTTTATAAAG
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501 CACCTGGCAA TTTTCCGTTA TTCAAAACAA TAACTATGCA ATTTCAACAC
551 CCAAGAANCA AGCNAACTGC TGCTGAAACA TTACTCAAAA ACCATTGCTG
601 TAGTTTTCCT GGTATCCAT

SEQ ID NO. 99
pMP506.reverse Length: 616 nt

1 CTTGCATGCC TGCAGGTCGA TCANCATGTT TAACAACAGG TACTAATAAT
51 CCTCTATCAG TGTCTGCTGC AATACCGATA TTCCAGTAAT GTTTATGAAC
101 GATTTCACCA GCTTCTTCAT TGAATGAAGT GTTAAGTGCT GGGTATTTTT
151 TCAATGCAGA AACAAGTGCT TTAACAACAT AAGGTAAGAA TGTTAACTTA
201 GTACCTTGTT CAGCTGCGAT TTCTTTAAAT TTCTTACGGT GATCCCATAA
251 TGCTTGAACA TCAATTTCAT CCATTAATGT TACATGAGGT GCAGTATGCT
301 TAGAGTTAAC CATTGCTTTC GCAATTGCTC TACGCATAGC AGGGATTTTT
351 TCAGTTGTTT CTGGGAAGTC GCCTTCTAAT GTTACTGCTG CAGGTGCTGC
401 AGGAGTTTCA GCAACTTCTT CACTTGTAGC TGAAGCAGCT GATTCATTTG
451 AAGCTGTTGG TGCACCACCA TTTAAGTATG CATCTACATC TTCTTTTGTA
501 ATACGACCAT TTTTTACCAG ATCCAGAAAC TGCTTTAATG TTTAACACCT
551 TTTTCACGTG CGTTATTTAC TTACTGAAGG CATTGCTTTA AACAGTCTGT
601 TTTCATCTAC TTCCTC

Mutant: NT437

phenotype: temperature sensitivity

Sequence map: Mutant NT437 is complemented by plasmid pMP652, which carries a 3.1 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 86; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal no significant similarities at this time. Current efforts are underway to complete the sequence contig and identify the essential gene contained in clone pMP652.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP652, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP652

SEQ ID NO. 100
pMP652.forward Length: 655 nt

```
  1 GTACCGGGGA TCGTCACTTA NCCTCTCTAT TTCAATTTCA ACTTATTTCG
 51 TCATCAAGTA TATGTGTTAT GCTTTTATAA CTTTGATTTC AATTCTATCA
101 ATATCTGTGA CATTGATAAC ATCGGACATA CGGTCTTCTT GTAACTTTTT
151 ATCCAATTCA AATGTATACT TTCCATAGTA TTTCTTTTTG ACTGTAATTT
201 TTCCTGTACT CATTTCACCG TAAAGACCAT AATTATCAAT AAGGTATTTT
251 CTTAATTTAA AATCAATCTC TTTCAATGAC ATCGCTTCTT TATCTATTTT
301 AAATGGGAAA AAGTCATAAT CATATTCACC AGTATGATCT TCTTTAATAA
351 CTCTTGCTTC TGCTATTAGG TCGACAGCTT TATCGTTTGC ACTCGTGATA
401 CCCCCAATAG AGTACTTTGC ACCTTCAAAT CTCTTATCCT CATTAACGTA
451 AAATATATTA AGAWTACGAW KKTACACCCG TATGATAATG TTTGCTTATC
501 TTTGCCAATT AAAGCAATAT TATTAACAGA ATTACCATCT ATGATATTCA
551 TAAATTTAAT ACTTGGTTGA ATGAAACTGG ATATAACCTG TCMCATTTTT
601 AATATTCMAT ACTAGGTTGA ATWATAATAA GCTTTTAATT TTTKGCTATT
651 TTCCC
```

SEQ ID NO. 101
pMP652.reverse Length: 650 nt

```
  1 GTCGACTCTA GAGGACTGCG TAATAACCTA TGAAAAATGA TATGAGCAAC
 51 GCCGCTCTGC TTTGCCGCAT ATACTAAATT TTCCACTTCA GGAATACGTT
101 TGAATGATGG ATGGATAATA CTTGGAATAA ACACAACGGT ATCCATTCCT
151 TTAAATGCTT CTACCATGCT TTCTTGATTA AAATAATCTA ATTGTCGAAC
201 AGGAACTTTT CCGCGCCAAT CTTCTGGAAC TTTCTCAACA TTTCTAACAC
251 CAATGTGAAA ATGATCTATG TGATTTGCAA TGGCTTGATT TGTAATATGT
301 GTGCCTAAAT GACCTGTAGC ACCTGTTAAC ATAATATTCA TTCACTTCAT
351 CTCCTAATCT TTATATACAT AACATAATAC TTATTTGATG GTTTTCAAAA
401 CATTTGATTT TATAAAAAAT TCTAATCTGT ATTTATTGTC GACGTGTATA
451 GTAAATACGT AAATATTANT AATGTTGAAA ATGCCGTAAT GACGCGTTTT
501 AGTTGATGTG TTTCACTAAT ATCATTGAAA ATTTTAATCA GGTACTACGA
551 CAATATGAAG TCTGTTTTGT GTCTGAAAAT TTTACAGTTT TTAAAATAAA
601 AATGGTATAA GTTGTGATTT GGTTTAAAAA ANAATCTCGA CGGATAANAA
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Mutant: NT438
phenotype: temperature sensitivity
Sequence map: : Mutant NT438 is complemented by plasmid pMP511, which carries a 2.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 87; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level

similarities to the *nifS* gene product, encoding a protein involved in the response pathway for nitrogen assimilation, from *A. azollae* (Genbank Accession No L34879; published in Jackman, D.M. *et al. Microbiology* **141**, pt.9 (1995) 2235-2244).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP511, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP511

SEQ ID NO. 102

pMP511 Length: 2341 nt

1	CTTGCATGCC	TGCAGGTCGA	TCTTTATTAT	NATCTACACC	ACGTANCATT
51	TCAACATGAC	CACGNTCATG	ACGATGTATG	CGTGCGTAAW	GTCCTGTKGY
101	WACATAATCK	GCACCTAAAT	TCATCGCATG	ATCTAAAAAG	GCTTTAAACT
151	TAATTTCTTT	ATWAMACATA	ACGTCTGGAT	TTGGAGTACG	ACCTTTTTTG
201	TATTCATCTA	AGAAATACGT	AAAGACTTTA	TCCCAATATT	CTTTTTCAAA
251	ATTAACAGCG	TAATACGGAA	TGCCAATTTG	ATTACACACT	TCAATAACAT
301	CGTTGTAATC	TTCAGTTGCA	GTACATACGC	CATTTTCGTC	AGTGTCATCC
351	CAGTTTTTCA	TAAATATGCC	AATGACATCA	TAACCTTGTT	CTTTTAAGAC
401	GTGGGCTGTT	ACAGAACTAT	CTACACCGCC	TGACATACCA	ACGACAACAC
451	GTTATATCTT	TATTTGACAA	TTATGACTCC	TCCTTAAATT	TAAAATATAT
501	TTTATGAATT	TCAGCTACAA	TTGCATTAAT	TTCATTTTCA	GTAGTCAATT
551	CGTTAAAACT	AAATCGAATC	GAATGATTTG	ATCGCTCCTC	ATCTTCGAAC
601	ATTGCATCTA	AAACATGCGA	CGGTTGTGTA	GAGCCTGCTG	TACATGCAGA
651	TCCAGACGAC	ACATAGATTT	GTGCCATATC	CAACAATGTT	AACATCGTTT
701	CAACTTCAAC	AAACGGAAAA	TATAGATTTA	CAATATGGCC	TGTAGCATCC
751	GTCATTGAAC	CATTTAATTC	AAATGGAATC	GCTCTTTCTT	GTAATTTAAC
801	TAAAAATTGT	TCTTTTAAAT	TCATTAAATG	AATATTGTTA	TCGTCTCGAT
851	TCTTTTCTGC	TAATTGTAAT	GCTTTAGCCA	TCCCAACAAT	TTGCGCAAGA
901	TTTTCAKTGC	CTAGCACGGC	GTTTCAATTC	TTGTTCACCG	CCAAGTTGAG
951	GATAATCTAG	TGTAACATGG	TCTTTAACTA	GTAATGCACC	GACACCTTTT
1001	GGTCCGCCAA	ACTTATGAGC	AGTAATACTC	ATTGCGTCGA	TCTCAAATTC
1051	GTCAAWCTTA	ACATCAAGAT	GTCCAATTGC	TTGAACCGCA	TCAACATGGA
1101	AATATGCATT	TGTCTCAGCA	ATAAATATCTT	GAATATCATA	AATTTGTTGC
1151	ACTGTGCCAA	CTTCATTATT	TACAAACATA	ATAGATACTA	AAATCGTCTT
1201	ATCTGTAATT	GTTTCTTCAA	GTTTGATCTA	AATCAATAGC	ACCTGTATCA
1251	TCARCATCTA	GATATGTTTA	CATCAAAACC	TYCTCGCTCT	AATTGTTCAA
1301	AAACATGTAA	CACAGAATGA	TGTTCAATCT	TCGATGTGAT	AATGTGATTA
1351	CCCAATTGTT	CATTTGCTTT	TACTATGCCT	TTAATTGCCG	TATTATTCGA

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1401 TTCTGTTGCG CCACTCGTAA ATATAATTTC ATGTGTATCT GCACCAAGTA
1451 ATTGTGCAAT TTGACGTCTT GACTCATCTA AATATTTACG CGCATCTCTT
1501 CCCTTAGCAT GTATTGATGA TGGATTACCA TAATGCGAAT TGTAAATCGT
1551 CATCATCGCA TCTACTAACT TCAGGTTTTA CTGGTGTGGT CGCAGCATAA
1601 TCTGCATAAA TTTCCCATGT TTGGACAACT CCTCACAATT TTATCAATGT
1651 TCCAATAATA GCACCTTAAC ATACTATTTT TCTAACTTTT CTGTTTAACT
1701 TTATTTATAA TGTTTTTAAT TATATTTTAC CATTTTCTAC ACATGCTTTT
1751 CGATAGGCTT TTTTAAGTTT ATCGCTTTAT TCTTGTCTTT TTTATAAATT
1801 TTAGTATTTG CAGATATTTT TTTATTTGTA AAATGTAACG TACTATTATT
1851 TTGGTTATGA GCAATTTAAT ATTTATCTGG TTATTCGGAT TGGTATACTT
1901 CTTATATCAT AAAAAAGGAA GGACGATATA AAAATGGCGG ATTAAATATT
1951 CAGCAKKRAA CCTTGTCCCT ATTCGAGAAG GTGAAGATGA ACAAACAGCA
2001 ATTAATAATA TGGTTAATCT CGCACAACAT TTAGACGAAT TATCATATGA
2051 AAGATATTGG ATTGCTGAAC ACCATAACGC TCCCAACCTA GTAAGTTCAG
2101 CAACTGCTTT ATTAATTCAA CATACGTTAG AACATACGAA ACACATACGT
2151 GTAGGTTCTG GAGGCATCAT GTTACCTAAT CATGCTCCAT TAATCGTTGC
2201 GGAACAATTT GGCACGATGG CAACATTATT TCCAAATCGT GTCGATTTAG
2251 GATTAGGACG TGCACCTGGA ACAGATATGA TGACCGCAAG TGCATTAAGA
2301 CGAGATCGAC TNTAGAGGAT CCCCGGGTAC CGAGCTCGAA T
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Mutant: NT462

phenotype: temperature sensitivity

Sequence map: : Mutant NT462 is complemented by plasmid pMP540, which carries a 2.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 88; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal limited peptide-level similarity to a transposase-like protein from *S. aureus;* the putative function of the ORF contained in clone pMP540 is unclear and will require further characterization.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP540, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP540

SEQ ID NO. 103

pMP540 Length: 2026 nt

```
   1 AAGGAAACCA CCAACACCTG CGCCAACTAA ACCKCCTGTT AGTGCAGAAA
  51 TAACGCTAAT AGCCCCCGCA CCTAAAGCAG CTRKNGTTTT TGTATATGCA
 101 GAAGAAAGAT ATAATGTTGC AGTATCTTTA CCTGTTTCTA CATATTGAGT
 151 TTTACCCGCT CTCAATTGGT CTTCAGCTTT ATATTTNTWT ATTTCTTCTW
 201 TAGTAAATAT ATCTTCCRGT TTATAACCTT TTTTCTCAAG TTCATCAAAT
 251 AAATTTWGGT TACTCAAATA TATTACCTTT GCTTGAGAAT GGTCTAACTT
 301 ATCTTCAGCA TGAGCTACAT CTGAATTATA GAGATAATGA AATTGGACTA
 351 ACAAATAATA CACCAGCAGC TRRTAATAAG AGATTTTTAA TTCGTTTTTC
 401 ATTAGTTTCT TTTAGATGAT TTTTGTATTT AGATTTCGTA TAAACAGAAA
 451 CTAGATTTTT TCATGATCGA CCTATCTTTT GTCCAGATAC AGTGAGACCT
 501 TGTCATTTAA ATGATTTTTA ATTCGTCTTG TACCAGAGAC TTTTCTATTA
 551 GAATTAAAAA TATTTATGAC GGCTGTTCTA TGTTTGAATC ATCTTTAGTG
 601 ATTTTATTAT CTTTTCTTTT TATAGAATCA TAATAGGTAC TTCTTAGTAT
 651 TATCAGGACT TTACACATTG NTGATACTGA ATANTGATGT GCATTCTTTT
 701 GAATGACTTC TATTTTTGCC CCATAATCAG CGCTACTTGC TTTAAAATAT
 751 CGTGCTCCAT TTTAAAATGT TGAACTTCTT TGCGTAATTT AATCAGGTCT
 801 TTTTCTTCAT CCGATAAGTT ATCTTGGTGA TTGAATGTAC CCGTGTTTTG
 851 ATGTTGCTTT ATCCATTTTC CTACATTTTA TAACCGCCAT TTACAAACGT
 901 CGAAKGTGTG AAATCATACT CGCGTWTAAT TTCATTCCTA GGCTTACCAT
 951 TTTTATATAA TCTAACCATT TGTAACTTAA ACTCTGAACT AAATGATCTT
1001 CTTTCTCTTG TCATAATAAA ATCGCCTACT TTCTTAAATT AACAATATCT
1051 ATTCTCATAG AATTTGTCCA ATTAAGTGTA GACGATTCAA TCTATCAGCT
1101 AGAATCATAT AACTTATCAG AAGCAAGTGA CTGTGCWTGT ATATTTGCCG
1151 MTGATATAAT AGTAGAGTCG CCTATCTCTC AGGCGTCAAT TTAGACGCAG
1201 AGAGGAGGTG TATAAGGTGA TGCTYMTTTT CGTTCAACAT CATAGCACCA
1251 GTCATCAGTG GCTGTGCCAT TGCGTTTTTY TCCTTATTGG CTAAGTTAGA
1301 CGCAATACAA AATAGGTGAC ATATAGCCGC ACCAATAAAA ATCCCCTCAC
1351 TACCGCAAAT AGTGAGGGGA TTGGTGTATA AGTAAATACT TATTTTCGTT
1401 GTCTTAATTA TACTGCTAAT TTTTCTTTTT GTAAAATATG CAAGGTTTTA
1451 AAGAGAAACA TCAAGAACTA AAAAAGGCTY TATGTCAAAT TGGACTGATG
1501 CGTTCAATAT CCGAAGTTAA GCAACTAAAC ATTGCTTAAC TTCCTTTTTA
1551 CTTTTTGGAG CGTAAAGTTT TGAACATAAT AATATTCGAT TGCGCAAATG
1601 ATTGTAACTT CCATAACCAA AAGATGTACG TTTAATTAAT TTTATTTTGT
1651 TATTTATACC TTCTAAAGGA CCATTTGATA AATTGTAATA ATCAATGGTT
1701 ACACTATTAA AAGTGTCACA AATTCTTATG AATCTGGCAT AAACTTTGAA
1751 TTAACTAAAT AAGTAAGAAA ACCTCGGCAC TTTATCATTT TAATAGTGTC
1801 GAGATTTTTA TAGATACTAC AAATATTTAT AACATAGTTA AACTCATCTA
1851 ATGACTTATA TTTTTGTTTC ATCACAATAT GAACAATTAT TTATTGGACG
1901 TATTTTGCTC TTTTTTTATT TCAGAAACTG ACTTAGGATT TTTATTAAAT
1951 TTTCTACCCA ATTCATCTGT ATAAGAAATA TCGGTATCAA ATTGAAAATC
2001 ATCAACAGAT CGACCTGCAG GCATGC
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Mutant: NT482

phenotype: temperature sensitivity

Sequence map: : Mutant NT482 is complemented by plasmid pMP560, which carries a 2.7 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 89. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarity at the peptide-level to the *folC* gene product, encoding folyl polyglutamate synthase (FGPS), from *B. subtilis* (Genbank Accession No. L04520; published in Mohan, S. *et al., J. Bacteriol.* **171** (1989) 6043-6051.)

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP560, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP560

SEQ ID NO. 104

pMP560 Length: 2736 nt

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  1 TGCCTGCAGG TCGATCTTCT ATGTAAATAA TCAAATGACG TTTCTTCTAT
 51 AGATATAAAT TGATATASAA AACTAAAAAT ACAACTGCAA CTATAAGATA
101 ACAATACTAC CAAATGACAA CCTCCTTATG TAAATTATAG TTAGTTATTA
151 CCAAAATGTA AATATACACT ATTTTTCAAG AATTGAACCG CTTTTTCATT
201 TAAATTTTTC AATATTGCTA AGCATAATTG ATGGATACTT TAACAACCCA
251 TTACTGCTCG GCAAAATTAA TAATGGCAAG AAATTGAACC TTATAAACAC
301 ATACGATTTA GAGCATAAAA AATAACCATG AAGCTCTACC TATTGATTAA
351 ATARATTCTT CATGGCTATT TTAGTTTTAG TTTTATAATG CTTCAAAGTC
401 TAATTTTGAT TTAACTTCAC TTATGAAATA CAGACTACCG GTAATTACTA
451 ATGTATCACC TTGATAATTT TTTATAAATT CAACGTAGTC ATCTACTAAT
501 TGTATTTCAT CATTTTCAAT ACTACCTACA ATTTCTTCTT TGCGTAACGC
551 TTTCGGAAAA TCAAATTCAG TTGCATAAAA CGTATGCGCA ATTAAACTTA
601 AATGTTTGAC CATCTCGTTA ATCGGTTTTC CGTTTATTGC TGASAACAAA
651 ATATCTACTT TTTCTTTATC ATGGTACTGT TTAATTGTAT CAATTAGAGC
701 ATCTATACTC TCTGAATTAT GYGCGCCATC CAAAATGATT AAAGGYTTGT
751 CATGCACCTG CTCAATACGT CCAGTCCAAC GAACTGATTC AATACCGTCT
801 ATCATCTTAT TGAAATCTAA TTCAATTAAT CCTTGTTCAT TTAATTCAAT
851 AAGAGCTGTT ATGGCTAATG CAGCAAWTTT GTTTCTGATG TTTCACCTAA
901 CATGCTTAAA ATGATTGTTT CTAATTCATA ATCTTTATAA CGGTAAGTTA
951 AATTCATCAT TTTGCGATAC AACAACAATT TCTCTATCTA ATTCAATGGC
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1001 TTTGCATGTT GTTCAATTGC GCGTTCACGA ACATATTTTA ATGCATCTTC
1051 ATTTTTTACA GCATATATCA CTGGAACKTT AGGSTTTATA ATCGCGCCYT
1101 TATCCCTAGC AATATCTAGA TAAGTACCAC CTAAAATATC TGTATGGTCT
1151 AGACCGATAC TAGTTAAGAT TGATAAAACC GGTGTAAAGA CATTTGTCGA
1201 ATCGTTCTTT ATACCCAATC CAGCCTCAAC AATGACAAAA TCAACAGGAT
1251 GTATTTCACC AAAATATAAA AACATCATCG CTGTGATTAT TTCGAATTCA
1301 GTTGCAAMMM CTAAATCTGT TTCAMSTTCC ATCATTTCAA TTAACTGGTT
1351 TAATACGTGA TACTAATTCT AACAATAGCG TCATTTGATA TTGGCAACAC
1401 CATTTAGRAT AATTCGTTCA TTAAATGTTT CAATAAACGG CGACGTAAAT
1451 GTACCTACTT CATAACCATT TTCAACTAAA GCTGTTCTAA GGTAAGCAAC
1501 TGTAGAGCCT TTACCATTTG TGCCACSKAC ATGAATACCC TTAATGWTAT
1551 TTTGAGGATT ATTAAATTGT GCTAGCATCC ATTCCATACG TTTAACACCT
1601 GGTTTGATGC CAAATTTAGT TCTTTCGTGT ATCCAATACA AGCTCTCTAG
1651 GTAATTCATT GTTACTAACT CCTATGCTTT TAATTGTTCA ATTCTTGCCT
1701 TCACACCATC ATATTTTTCT TGATAATCTT GTTTTTTACG TTTTTCTTCA
1751 TTTATAACCT TTTCAGGTGC TTTACTTACA AAGTTTTCAT TAGAGAGCTT
1801 TTTATCTACT CTATCTAATT CGCTTTGAAG TTTAGCTAAT TCTTTTTCCA
1851 AACGGCTGAT TTCCTTATCC ATATCAATTA GCCCTTCTTA ATGGTAATAC
1901 CCACTTTACC TGCAATTACA ACTGATGTCA TTGCTTTCTC AGGAATTTCC
1951 AACGTCAGTG CTAATATTTA AGGTACTAGG ATTACAGAAT TTGATTAAAT
2001 AATCTTTGTT TTGTGATAAA GTTGTTTCAA TTTCTTTATC TTTAGCTTGA
2051 ATTAAAATAG GTATTTCTTT AGACAATGGC GTATTTACTT CTACACGTGA
2101 TTGTCTTACA GATTTAATGA TTTCAACAAG TGGTKGCATT GTTTGTTAAC
2151 TTTCTTCAAA AATCAATGAT TCACGCACTT CTGGCCATGA AGCTTTAACA
2201 ATTGTGTCAC CTTCATGTGG TAAACTTTGC CATATTTTCT CTGTTACAAA
2251 TGGCATGAAT GGATGTAGCA TTCTCATAAT ATTGTCTAAA GTATAACTCA
2301 ATACTGAACG TGTAACTTGT TTTTGTTCTT CATCATTACT ATTCATTGGA
2351 ATTTTACTCA TTTCAATGTA CCAATCACAG AAATCATCCC AAATGAAATT
2401 ATATAATGCA CGTCCAACTT CGCCGAATTC ATATTTGTCA CTTAAATCAG
2451 TAACTGTTGC AATCGTTTCA TTTAAACGTG TTAGAATCCA TTTATCTGCT
2501 AATGATAAGT TACCACTTAA ATCGATATCT TCAACTTTAA AGTCTTCACC
2551 GATATTCATT AAACTGAAAC GTGCCCCATT CCAGATTTTA TTGATAAAGT
2601 TCCACACTGA CTCAACTTTT TCAGTTGAGT ATCTTAAATC ATGTCCTGGA
2651 GATGAACCTG TTGCTAAGAA GTAACGCAAG CTATCAGCAC CGTATTCGTC
2701 AATAACATCC ATTGGATCGA CCTGCAGGCA TGCAAG
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Mutant: NT486

phenotype: temperature sensitivity

Sequence map: : Mutant NT486 is complemented by plasmid pMP567, which carries a 2.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 90; no apparent restriction sites for EcoR I, HinD III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to the *accA* gene product, encoding the alpha

subunit of acetyl-CoA-carboxylase carboxyl transferase (EC 6.4.1.2), from *B. stearothermophilus* (Genbank Accession No. D13095); this gene product forms part of an enzyme complex responsible for fatty acid biosynthesis and is thought to be essential.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP567, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP567

SEQ ID NO. 105
pMP567 Length: 2255 nt

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   1 CNCGNNAGCG ANGTNGCCGA GGATCCTCTA GAGTCNATCG GTTATCGGTG
  51 AAAAGATATG TCGCATCATT GATTACTGCA CTGAGAACCG TTTACCATTT
 101 ATTCTTTTCT CTGCAAGTGG TGGTGCACGT ATGCAAGAAG GTATTATTTC
 151 CTTGATGCAA ATGGGTAAAA CCAGTGTATC TTTAAAACGT CATTCTGACG
 201 CTGGACTATT ATATATATCA TATTTAACAC ATCCAACTAC TGGTGGTGTA
 251 TCTGCAAGTT TTGCATCAGT TGGTGATATA AATTTAAGTG AGCCAAAAGC
 301 GTTGATAGGT TTTGCAGGTC GTCGAGTTAT TGAACAGACA ATAAACGAAA
 351 AATTGCCAGA TGATTTCCAA ACTGCAGAAT TTTTATTAGA GCATGGACAA
 401 TTGGATAAAG TTGTACATCG TAATGATATG CGTCAAACAT TGTCTGAAAT
 451 TCTAAAAATC CATCAAGAGG TGACTAAATA ATGTTAGATT TTGAAAAACC
 501 ACTTTTTGAA ATTCGAAATA AAATTGAATC TTTAAAAGAA TCTCAAGATA
 551 AAAATGATGT GGATTTACCA AAGAAGAATT TGACATGCCT TGAARCGTCM
 601 TTGGRACGAG AAACTAAAAA AATATATACA AATCTAAAAC CATGGGATCG
 651 TGTGCAAATT GCGCGTTTGC AAGAAAGACC TACGACCCTA GATTATATTC
 701 CATATATCTT TGATTCGTTT ATGGAACTAC ATGGTGATCG TAATTTTAGA
 751 GATGATCCAG CAATGATTGG TGGTATTGGC TTTTTAAATG GTCGTGCTGT
 801 TACAGTYRTK GGACAACAAC GTGGAAAAGA TACWAAAGAT RATATTTATC
 851 GAAATTTTKG GTATGGCGCA TCCAGAAGGT TATCGAAAAG CATTACGTTT
 901 AATGAAACAA GCTGAAAAAT TCAATCGTCC TATCTTTACA TTTATAGATA
 951 CAAAAGGTGC ATATCCTGGT AAAGCTGCTG AAGAACGTGG ACAAAGTGAA
1001 TCTATCGCAA CAAATTTGAT TGAGATGGCT TCATTAAAAG TACCAGTTAT
1051 TGCGATTGTC ATTGKYGAAG GTGGCAGTGG AGGTGCTCTA GGTATTGGTA
1101 TTGCCAATAA AGYATTGATG TTAGAGAATA GTACTTACTC TGWTATATCT
1151 CCTGAAGGTG CAGCGGCATT ATTATGGAAA GACAGTAATT TGGCTAAAAT
1201 YGCAGCTGAA ACAATGAAWA TTACTGCCCA TGATATTAAG CAATTAGGTA
1251 TTATAGATGA TGYCATTTCT GAACCACTTG GCGGTGCACA TAAAGATATT
1301 GAACAGCAAG CTTTAGCTAT TAAATCAGCG TTTGTTGCAC AGTTAGATTC
1351 ACTTGAGTCA TTATCAACGT GATGAAATTG CTAATGATCG CTTTGAAAAA
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1401 TTCAGAAATA TCGGTTCTTA TATAGAATAA TCAACTTGAG CATTTTTATG
1451 TTAAATCGAT ACTGGGTTTT ACCATAAATT GAAGTACATT AAAACAATAA
1501 TTTAATATTT AGATACTGAA TTTTTAACTA AGATTAGTAG TCAAAATTGT
1551 GGCTACTAAT CTTTTTTTAA TTAAGTTAAA ATAAAATTCA ATATTTAAAA
1601 CGTTTACATC AATTCAATAC ATTAGTTTTG ATGGAATGAC ATATCAATTT
1651 GTGGTAATTT AGAGTTAAAG ATAAATCAGT TATAGAAAGG TATGTCGTCA
1701 TGAAGAAAAT TGCAGTTTTA ACTAGTGGTG GAGATTCACC TGGAATGAAT
1751 GCTGCCGTAA GAGCAGTTGT TCGTACAGCA ATTTACAATG AAATTGAAGT
1801 TTATGGTGTG TATCATGGTT ACCAAGGATT GTTAAATGAT GATATTCATA
1851 AACTTGAATT AGGATCRAGT TGGGGATACG ATTCAGCGTG GAGGTACATT
1901 CTTGTATTCA GCAAGATGTC CAGAGTTTAA GGAGCAAGAA GTACGTAAAG
1951 TTGCAATCGA AAACTTACGT AAAAGAGGGA TTGAGGGCCT TGTAGTTATT
2001 GGTGGTGACG GTAGTTATCG CGGTGCACAA CGCATCAGTG AGGAATGTAA
2051 AGAAATTCAA ACTATCGGTA TTCCTGGTAC GATTGACAAT GATATCAATG
2101 GTACTGATTT TACAATTGGA TTTGACACAG CATTAAATAC GATTATTGGC
2151 TTAGTCGACA AAATTAGAGA TACTGCGTCA AGTCACGCAC GAACATTTAT
2201 CATTGAAGCA ATGGGCCGTG ATTGTGGAGT CATCTGGAGT CGACCTGCTA
2251 GTCTT

II. Homologous Genes

As described above, the use of genes from other pathogenic bacterial strains and species which are homologous to the identified genes from *Staphylococcus aureus* is also provided. Such homologous genes not only have a high level of sequence similarity with the particular *S. aureus* genes, but also are functional equivalents. This means that the gene product has essentially the same biological activity. Therefore, the homologous genes are identifiable, for example, based on a combination of hybridization of all or a portion of one gene to its homologous counterpart, and the ability of the homologous gene to complement the growth conditional mutant of *S. aureus* under non-permissive conditions. The ability of the homologous gene to hybridize with sequences from the *S. aureus* gene provides that homologous gene using generally accepted and used cloning techniques. The ability of the homologous gene to complement a defective *S. aureus* gene demonstrates that the genes are essentially equivalent genes found in different bacteria.

Specific examples of methods for identifying homologous genes are described in Van Dijl et al., U.S. Patent 5,246,838, issued September 21, 1993. In addition to the direct hybridization methods for identifying and isolating homologous genes mentioned above, Van Dijl et al. describe the isolation of homologous genes by isolating clones of a host bacterial strain which contain random DNA fragments from a donor microorganism. In those clones a

specific host gene has been inactivated (such as by linkage with a regulatable promoter), and inserted homologous genes are identified by the complementation of the inactivated gene function. Homologous genes identified in this way can then be sequenced.

If the function of the product of a specific host gene is known, homologous gene products can often be isolated (by assaying for the appropriate activity) and at least partially sequenced (*e.g.*, N-terminal sequencing). The amino acid sequence so obtained can then be used to deduce the degenerate DNA base sequence, which can be used to synthesize a probe(s) for the homologous gene. A DNA library from another microorganism is then probed to identify a clone(s) containing a homologous gene, and the clone insert sequenced.

These and other methods for identifying homologous genes are well-known to those skilled in the art. Therefore, other persons can readily obtain such genes which are homologous to the genes corresponding to SEQ ID NO. 1-105.

III. Evaluation of Gene as Therapeutic Target

A. General Considerations

While the identification of a particular bacterial gene as an essential gene for growth in a rich medium characterizes that gene as an antibacterial target, it is useful to characterize the gene further in order to prioritize the targets. This process is useful since it

allows further work to be focused on those targets with the greatest therapeutic potential. Thus, target genes are prioritized according to which are more likely to allow identification of antibacterial agents which are:

1. Highly inhibitory to the target in relevant pathogenic species;

2. Cause rapid loss of bacterial viability;

3. Not have frequently arising resistance mechanisms;

4. Have high selectivity for the bacterial target and little, or preferably no, effect on the related mammalian targets;

5. Have low non-specific toxicity to mammals; and

6. Have appropriate pharmacodynamic and physical properties for use as a drug.

Consequently, target genes are prioritized using a variety of methods, such as those described below.

B. Methods for Recognizing Good Targets

Essential genes can be characterized as either bactericidal or bacteriostatic. Earlier work with *Salmonella* mutants established that the bactericidal/bacteriostatic distinction was a characteristic of inhibition of the specific gene, rather than of a mutant allele, and could be characterized *in vitro*. (Schmid et al., 1989, *Genetics* 123:625-633.) Therefore, preferred targets (high priority) are those which are highly bactericidal when inhibited, causing cell death. A subset of the bactericidal essential genes can be identified as

strongly bactericidal, resulting in rapid cell death when inhibited.

In *S. typhimurium*, inhibition of strongly bactericidal genes was shown to result in one of the following effects:

1. Cell lysis (such genes generally involved in cell wall biosynthesis);

2. Inhibition of protein synthesis;

3. DNA degradation; or

4. Entry into non-recoverable state involving cell cycle related genes.

In vivo switch

In addition to the prioritization of gene targets based on the observed *in vitro* phenotypes, further evaluation of a specific gene as a potential therapeutic target is performed based on the effects observed with loss of that gene function *in vivo*. One approach is the use of null mutants in which the mutant gene product is inactive at 37°C. In the case of essential genes for which temperature sensitive mutants were previously isolated, those mutant strains can be used in this evaluation if the gene product is essentially inactive at 37°C. If such a temperature sensitive mutant has not previously been isolated but a complementing clone of some growth conditional mutant is available, then the required null mutants can generally be isolated through the use of localized mutagenesis techniques (Hong and Ames, 1971, *Proc. Natl. Acad. Sci. USA* 68:3158-3162). The evaluation then involves the comparison of the

in vivo effects of the normal strain and the mutant strain. The comparison involves determinations of the relative growth *in vivo*, relative bactericidal phenotype *in vivo* and differences in response in various infection models.

In addition to gene target evaluations using null mutant experiments, related evaluations can be performed using "*in vivo* switch" methods. Such methods allow control of the expression of a gene *in vivo*, and so provide information on the effects of inhibiting the specific gene at various time points during the course of an infection in a model infection system. In effect, an *in vivo* switch provides a mimic of the administration of an inhibitor of a gene, even if such an inhibitor has not yet been identified.

Such *in vivo* switch methods can be carried out by using recombinant strains of a pathogenic bacterium, which carry a test gene transcriptionally linked with an artificially controllable promoter. One technique for doing this is to use the natural promoter for the test gene, and insert an operator site in a position so that transcription will be blocked if a repressor molecule is bound to the operator. Expression of the repressor molecule is then placed under artificial control by linking the gene for the repressor with a promoter which can be controlled by the addition of a small molecule. For example, a β-lactamase receptor/repressor/promoter system can be used to control expression of a *lac* repressor, which, in turn, will bind to a *lac* operator site inserted in the test gene. These DNA constructs are then inserted into bacteria in which the

endogenous copy of the test gene has been inactivated, and those bacteria are used in various infection models. Therefore, for this system, the test gene will be expressed prior to administration of a β-lactam. However, when a β-lactam with little or no intrinsic antibacterial activity (*e.g.*, CBAP) is administered to an animal infected with the recombinant bacteria, the β-lactam induces production of lac repressor. The lac repressor molecule then binds to the lac operator, stopping (turning off) expression of the test gene.

The method can be extended by administering the β-lactam (or other appropriate controller molecule) at different times during the course of an infection, and/or according to different schedules of multiple dosing. Also, many different designs of *in vivo* switch may be used to provide control over the test gene. In general, however, such a method of target evaluation provides information such as:

1. a measure of the "cidalness" of the target gene following inhibition of that gene;

2. a benchmark against which to measure chemical inhibitors as they are identified, since the *in vivo* switch can mimic complete inhibition of the gene;

3. an estimate of the efficacy of inhibitor use at different time points in an infection process; and

4. an estimate of the efficacy of inhibitor use in various types of infections, in various *in vivo* environments.

Information of this nature is again useful for focusing on the gene targets which are likely to be the best therapeutic targets.

C. *In vivo* evaluation of microbial virulence and pathogenicity

Using gene target evaluation methods such as the null mutant and *in vivo* switch methods described above, the identified target genes are evaluated in an infection model system. (References herein to the use of animals or mammals should be understood to refer to particular infection models. Other infection systems may be used, such as cell-based systems as surrogates for whole organism models, or systems to evaluate possible antimicrobial targets of pathogens of organisms other than animals (*e.g.*, plants). The criteria for evaluation include the ability of the microbe to replicate, the ability to produce specific exoproducts involved in virulence of the organism, and the ability to cause symptoms of disease in the animals.

The infection models, *e.g.*, animal infection models, are selected primarily on the basis of the ability of the model to mimic the natural pathogenic state of the pathogen in an organism to be treated and to distinguish the effects produced by activity or by loss of activity of a gene product (*e.g.*, a switch in the expression state of the gene). Secondarily, the models are selected for efficiency, reproducibility, and cost containment. For mammal models, rodents, especially mice, rats, and rabbits, are generally the preferred species. Experimentalists have the greatest

experience with these species. Manipulations are more convenient and the amount of materials which are required are relatively small due to the size of the rodents.

Each pathogenic microbe (*e.g.*, bacterium) used in these methods will likely need to be examined using a variety of infection models in order to adequately understand the importance of the function of a particular target gene.

A number of animal models suitable for use with bacteria are described below. However, these models are only examples which are suitable for a variety of bacterial species; even for those bacterial species other models may be found to be superior, at least for some gene targets and possibly for all. In addition, modifications of these models, or perhaps completely different animal models are appropriate with certain bacteria.

Six animal models are currently used with bacteria to appreciate the effects of specific genes, and are briefly described below.

1. <u>Mouse Soft Tissue Model</u>

The mouse soft tissue infection model is a sensitive and effective method for measurement of bacterial proliferation. In these models (Vogelman et al., 1988, *J. Infect. Dis.* 157: 287-298) anesthetized mice are infected with the bacteria in the muscle of the hind thigh. The mice can be either chemically immune compromised (*e.g.*, cytoxan treated at 125 mg/kg on days -4, -2, and 0) or immunocompetent. The dose of microbe necessary to cause an

infection is variable and depends on the individual microbe, but commonly is on the order of 10^5 - 10^6 colony forming units per injection for bacteria. A variety of mouse strains are useful in this model although Swiss Webster and DBA2 lines are most commonly used. Once infected the animals are conscious and show no overt ill effects of the infections for approximately 12 hours. After that time virulent strains cause swelling of the thigh muscle, and the animals can become bacteremic within approximately 24 hours. This model most effectively measures proliferation of the microbe, and this proliferation is measured by sacrifice of the infected animal and counting colonies from homogenized thighs.

2. Diffusion Chamber Model

A second model useful for assessing the virulence of microbes is the diffusion chamber model (Malouin et al., 1990, *Infect. Immun.* 58: 1247-1253; Doy et al., 1980, *J. Infect. Dis.* 2: 39-51; Kelly et al., 1989, *Infect. Immun.* 57: 344-350. In this model rodents have a diffusion chamber surgically placed in the peritoneal cavity. The chamber consists of a polypropylene cylinder with semipermeable membranes covering the chamber ends. Diffusion of peritoneal fluid into and out of the chamber provides nutrients for the microbes. The progression of the "infection" can be followed by examining growth, the exoproduct production or RNA messages. The time experiments are done by sampling multiple chambers.

3. Endocarditis Model

For bacteria, an important animal model effective in assessing pathogenicity and virulence is the endocarditis model (J. Santoro and M.E. Levinson, 1978, *Infect. Immun.* 19: 915-918). A rat endocarditis model can be used to assess colonization, virulence and proliferation.

4. Osteomyelitis Model

A fourth model useful in the evaluation of pathogenesis is the osteomyelitis model (Spagnolo et al., 1993, *Infect. Immun.* 61: 5225-5230). Rabbits are used for these experiments. Anesthetized animals have a small segment of the tibia removed and microorganisms are microinjected into the wound. The excised bone segment is replaced and the progression of the disease is monitored. Clinical signs, particularly inflammation and swelling are monitored. Termination of the experiment allows histolic and pathologic examination of the infection site to complement the assessment procedure.

5. Murine Septic Arthritis Model

A fifth model relevant to the study of microbial pathogenesis is a murine septic arthritis model (Abdelnour et al., 1993, *Infect. Immun.* 61: 3879-3885). In this model mice are infected intravenously and pathogenic organisms are found to cause inflammation in distal limb joints. Monitoring of the inflammation and comparison of inflammation vs. inocula allows assessment of the virulence of related strains.

6. Bacterial Peritonitis Model

Finally, bacterial peritonitis offers rapid and predictive data on the virulence of strains (M.G. Bergeron, 1978, *Scand. J. Infect. Dis.* Suppl. 14: 189-206; S.D. Davis, 1975, *Antimicrob. Agents Chemother.* 8: 50-53). Peritonitis in rodents, preferably mice, can provide essential data on the importance of targets. The end point may be lethality or clinical signs can be monitored. Variation in infection dose in comparison to outcome allows evaluation of the virulence of individual strains.

A variety of other *in vivo* models are available and may be used when appropriate for specific pathogens or specific genes. For example, target organ recovery assays (Gordee et al., 1984, *J. Antibiotics* 37:1054-1065; Bannatyne et al., 1992, *Infect.* 20:168-170) may be useful for fungi and for bacterial pathogens which are not acutely virulent to animals. For additional information the book by Zak and Sande (EXPERIMENTAL MODELS IN ANTIMICROBIAL CHEMOTHERAPY, O. Zak and M.A. Sande (eds.), Academic Press, London (1986) is considered a standard.

It is also relevant to note that the species of animal used for an infection model, and the specific genetic make-up of that animal, may contribute to the effective evaluation of the effects of a particular gene. For example, immuno-incompetent animals may, in some instances, be preferable to immuno-competent animals. For example, the action of a competent immune system may, to some degree, mask the effects of altering the level of activity of the test gene product as compared to a similar infection in an

immuno-incompetent animal. In addition, many opportunistic infections, in fact, occur in immuno-compromised patients, so modeling an infection in a similar immunological environment is appropriate.

In addition to these *in vivo* test systems, a variety of *ex vivo* models for assessing bacterial virulence may be employed (Falkow et al., 1992, *Ann. Rev. Cell Biol.* 8:333-363). These include, but are not limited to, assays which measure bacterial attachment to, and invasion of, tissue culture cell monolayers. With specific regard to *S. aureus*, it is well documented that this organism adheres to and invades cultured endothelial cell monolayers (Ogawa et al., 1985, *Infect. Immun.* 50: 218-224; Hamill et al., 1986, *Infect. and Imm.* 54:833-836) and that the cytotoxicity of ingested *S. aureus* is sensitive to the expression of known virulence factors (Vann and Proctor, 1988, *Micro. Patho.* 4:443-453). Such *ex vivo* models may afford more rapid and cost effective measurements of the efficacy of the experiments, and may be employed as preliminary analyses prior to testing in one or more of the animal models described above.

IV. Screening Methods for Antibacterial Agents

A. Use of Growth Conditional Mutant Strains

1. Hypersensitivity and TS Mutant Phenoprints

In addition to identifying new targets for drug discovery, the growth conditional mutants are useful for screening for inhibitors of the identified targets, even before the novel genes or biochemical targets are fully

characterized. The methodology can be whole-cell based, is more sensitive than traditional screens searching for strict growth inhibitors, can be tuned to provide high target specificity, and can be structured so that more biological information on test compounds is available early for evaluation and relative prioritization of hits.

Certain of the screening methods are based on the hypersensitivity of growth conditional mutants. For example, conditionally lethal ts mutants having temperature sensitive essential gene functions are partially defective at a semi-permissive temperature. As the growth temperature is raised, the mutated gene causes a progressively crippled cellular function. It is the inherent phenotypic properties of such ts mutants that are exploited for inhibitor screening.

Each temperature sensitive mutant has secondary phenotypes arising from the genetic and physiological effects of the defective cellular component. The genetic defect causes a partially functional protein that is more readily inhibited by drugs than the wild type protein. This specific hypersensitivity can be exploited for screening purposes by establishing "genetic potentiation" screens. In such screens, compounds are sought that cause growth inhibition of a mutant strain, but not of wild type, or greater inhibition of the growth of a mutant strain than of a wild type strain. Such compounds are often (or always) inhibitors of the wild type strain at higher concentrations.

Also, the primary genetic defect can cause far-reaching physiological changes in the mutant cells, even in semi-permissive conditions. Necessity for full function of biochemically related proteins upstream and downstream of the primary target may arise. Such effects cause hypersensitivity to agents that inhibit these related proteins, in addition to agents that inhibit the genetically defective cellular component. The effects of the physiological imbalance will occur through metabolic interrelationships that can be referred to as the "metabolic web". Thus, in some cases, the initial genetic potentiation screen has the ability to identify inhibitors of either the primary target, or biochemically related essential gene targets.

With sufficient phenotypic sensors, a metabolic fingerprint of specific target inhibition can be established. Therefore, the mutant strains are evaluated to identify a diverse repertoire of phenotypes to provide this phenotypic fingerprint, or "phenoprint". These evaluations include hypersensitivities to known toxic agents and inhibitors, carbon source utilization, and other markers designed to measure specific or general metabolic activities for establishing a mutant phenoprint that will aid in interpretation of inhibitor profiles.

2. Determination of hypersusceptibility profiles

As an illustration of the hypersusceptibility profiles for a group of bacterial ts mutant strains, the minimal inhibitory concentrations (MICs) of various drugs

and toxic agents were determined for a set of *Salmonella typhimurium* temperature-sensitive essential gene mutants.

The MICs were measured by using a standard micro broth dilution technique following the recommendations of the National Committee for Clinical Laboratory Standards (1994). Bacteria were first grown in Mueller-Hinton broth at 30°C, diluted to 10^5 cfu/ml and used to inoculate 96-microwell plates containing two-fold dilutions of antibiotics in Mueller-Hinton broth. Plates were incubated for 20h at a semi-permissive temperature (35°C) and the MIC was determined as the lowest dilution of antibiotic preventing visible growth.

A two-fold difference in the susceptibility level of the mutant strain compared to that of the parental strain is within the limits of the experimental variation and thus a ≥4-fold decrease in MIC was considered as a significant hypersusceptibility.

Example 1: Hypersensitivity of *S. aureus secA* mutants

The *secA* mutant strain NT65 was found to be more sensitive to compound MC-201,250. The MIC of this compound on NT65 is 0.62 μg/ml and that on the wild type strain is 50 μg/ml. The inhibitory effect of MC-201,250 on *secA* mutants increased as screening temperatures increased. Other *secA* mutants, which may represent different alleles of the gene, are also hypersensitive to this compound by varying degrees, examples are shown in Table 1 below.

Table 1
Hypersensitivity of *secA* Alleles to MC201,250

Strain	MIC (μg/ml)
NT65	0.62
NT328	1.25
NT74	2.5
NT142	5
NT15	10
NT67	10
NT122	10
NT112	20
NT368	20
NT413	20
Wild Type (WT)	50

Furthermore, introduction of the wild type *secA* allele into NT65 raised the MIC to the wild type level. These data suggest that the hypersensitivity results from the *secA* mutation in the mutants.

To further demonstrate that the hypersensitivity to MC-201,250 is due to the *secA* mutation that causes the temperature sensitivity, heat-resistant revertants, both spontaneous and UV-induced, were isolated from NT65 and tested for their responses to the compound. In a parallel experiment, MC-201250-resistant revertants were also isolated from NT65 and tested for their growth at nonpermissive temperatures. The results showed that revertants able to grow at 43°C were all resistant to MC-201250 at the wild type level (MIC=50 μg/ml) and vice versa. Revertants able to grow at 39°C but not at 43°C showed intermediate resistance to MC-201,250 (MIC=1.25-2.5 μg/ml and vice versa The correlation between the heat-

sensitivity and MC-201,250-sensitivity strongly suggests that the *secA* gene product may be the direct target for MC-201,250.

The benefits of using hypersensitive mutants for screening is apparent, as this inhibitor would have not been identified and its specificity on *secA* would have not been known if wild type cells rather than the mutants were used in whole cell screening at a compound concentration of 10 μg/ml or lower.

Example 2: Hypersensitivity of *S. typhimurium gyr* mutants

The specific hypersensitivity of temperature sensitive mutations in a known target to inhibitors of that target is shown in Figure 1 with the susceptibility profile of three ts *S. typhimurium* mutant alleles of the gyrase subunit A (*gyrA212*, *gyrA215* and *gyrA216*) grown at a semi-permissive temperature (35°C). The graph shows the fold-increases in susceptibility to various characterized antibacterial agents compared to that observed with the wild-type parent strain. The data demonstrate the highly specific hypersusceptibility of these mutants to agents acting on DNA gyrase. Susceptibility to other classes of drug or toxic agents is not significantly different from the parent strain (within 2-fold).

In addition, different mutant alleles show unique hypersensitivity profiles to gyrase inhibitors. Coumermycin inhibits the B-subunit of the gyrase, while norfloxacin,

ciprofloxacin, and nalidixic acid inhibit the A-subunit. One mutant shows hypersusceptibility to coumermycin *(gyrA216)*, one to coumermycin and norfloxacin *(gyrA215)*, and another to norfloxacin and ciprofloxacin *(gyrA212)*. Note that a mutation in the gyrase subunit A (*gyrA215*) can cause hypersensitivity to B-subunit inhibitors and could be used to identify such compounds in a screen. In addition, some *gyrA* mutant strains show no hypersensitivity to known inhibitors; potentially, these strains could be used to identify novel classes of gyrase inhibitors. Overall these results show that a selection of mutated alleles may be useful to identify new classes of compounds that affect gyrase function including structural subunit-to-subunit interactions. Thus, use of the properties of the crippled gyrase mutants in a screen provides a great advantage over biochemical-based screens which assay a single specific function of the target protein *in vitro*.

Example 3: Hypersensitivity profiles of *Salmonella* ts mutants

Demonstration of the generalized utility of hypersensitive screening with the conditional lethal mutants has been obtained (Figure 2) by collecting hypersensitivity profiles from partly characterized *Salmonella* conditional ts mutants. The table shows the increased susceptibility of the mutant strains to various characterized antibacterial agents compared to the wild-type parent strain. A two-fold difference in the susceptibility level is within the limits

of the experimental variation and thus a ≥4-fold difference is significant.

A variety of hypersusceptibility profiles is observed among the ts mutants. These profiles are distinct from one another, yet mutants with related defects share similar profiles. The *parF* mutants, which have mutations closely linked to the *Salmonella* topoisomerase IV gene, are hypersusceptible to gyrase subunit B inhibitors (black circle), although these mutants are also susceptible to drugs affecting DNA or protein metabolism. Similarly, specificity within the hypersusceptibility profiles of two out of four ts mutants (SE7583, SE7587, SE5119 and SE5045) having possible defects in the cell wall biosynthesis machinery are also observed (mutants *dapA* and *murCEFG*, black diamond). The latter mutants are also susceptible to other agents and share their hypersusceptibility profile with a mutant having a defect in the incorporation of radioactive thymidine (SE5091).

Thus, the hypersensitivity profiles actually represent recognizable interrelationships between cellular pathways, involving several types of interactions as illustrated in Fig. 3. The patterns created by these profiles become signatures for targets within the genetic/metabolic system being sensitized. This provides a powerful tool for characterizing targets, and ultimately for dereplication of screening hits. The hypersusceptibility profiles have been established for 120 *Salmonella* and 14

Staphylococcus aureus ts mutants with a selection of 37 known drugs or toxic agents

The growth conditional mutants are also used in gene sensor methodology, *e.g.*, using carbon utilization profiles. Ts mutants fail to metabolize different carbon sources in semi-permissive growth conditions. The carbon sources not utilized by a specific mutant or group of mutants provide additional phenotypes associated with the crippled essential function. Moreover, some of these carbon source markers were also not used by the wild type strain exposed to sub-MIC concentrations of known drugs affecting the same specific cellular targets or pathways. For example, a sublethal concentration of cefamandole prevented the *Salmonella* wild type parent strain from metabolizing the same carbon source that was not used by either the *dapA* or the *murCEFG* mutant.

In combination, interrelationships within and between essential cellular pathways are manifested in hypersensitivity and biosensor profiles that together are employed for highly discriminatory recognition of targets and inhibitors. This information provides recognition of the target or pathway of compound action.

B. Screening Strategy and Prototypes

1. Strain Validation and Screening Conditions

Hypersensitive strains (not growth conditional) have been successfully used in the past for discovery of new drugs targeting specific cellular pathways. (Kamogashira and Takegata, 1988, *J. Antibiotics* 41:803-806; Mumata et

al., 1986, *J. Antibiotics* 39:994-1000.) The specific hypersensitivities displayed by ts-conditional mutants indicates that use of these mutants in whole cell screening provides a rapid method to develop target-specific screens for the identification of novel compounds. However, it is beneficial to eliminate mutants that will not be useful in semi-permissive growth conditions. Such mutant alleles may have nearly wild type function at the screening assay temperature. The simplest method for validating the use of ts mutants is to select those which show a reduced growth rate at the semi-restrictive growth temperature. A reduced growth rate indicates that the essential gene function is partially defective. More specific methods of characterizing the partial defect of a mutant strain are available by biochemical or physiological assays.

2. <u>Multi-Channel Screening Approach</u>

The phenoprint results above, demonstrate that ts mutants show specific hypersusceptibility profiles in semi-permissive growth conditions. As a screening tool, the mutant inhibition profile characterizes the effects of test compounds on specific bacterial pathways. Because the mutants are more sensitive than wild type strains, compounds with weak inhibition activity can be identified.

An example of a multi-channel screen for inhibitors of essential genes is shown in Fig. 4. In this screen design, one plate serves to evaluate one compound. Each well provides a separate whole-mutant cell assay (i.e., there are many targets per screening plate). The assays are

genetic potentiation in nature, that is, ts-hypersensitive mutants reveal compounds that are growth inhibitors at concentrations that do not inhibit the growth of the wildtype strain. The profile of mutant inhibition provides insight into the compound's target of inhibition. The ts mutants are grouped by their hypersensitivity profiles to known drugs or by their related defective genes. The figure illustrates the hypothetical growth inhibition results (indicated by "–") that would be obtained with a new antibacterial agent targeting DNA/RNA metabolism.

Different multi-channel screen designs can fit specific needs or purposes. The choice of a broadly-designed screen (such as in Fig. 4), or one focused on specific cellular pathways, or even specific targets can be made by the appropriate choice of mutants. More specific screen plates would use mutants of a specific gene target like DNA gyrase, or mutants in a specific pathway, such as the cell division pathway.

The use of the 96-well multi-channel screen format allows up to 96 different assays to characterize a single compound. As shown in Fig. 5, this format provides an immediate characterization or profile of a single compound. The more traditional format, using up to 96 different compounds per plate, and a single assay can also be readily accommodated by the genetic potentiation assays.

In comparing the two formats, the multi-channel screen format is generally compound-focused: prioritization of compounds run through the screen will occur, as decisions

are made about which compounds to screen first. Each plate provides an immediate profile of a compound. The more traditional format is target-focused: prioritization of targets will occur, as decisions are made about the order of targets or genetic potentiation screens to implement.

In a preferred strategy for screening large compound libraries, a "sub-library" approach is taken. In this approach, the compound library is divided into a number of blocks or "sub-libraries". All of the selected ts mutants are screened against one block of the compounds. The screen is carried out in 96-well plates and each plate serves to test 80 compounds (one compound per well) on one mutant strain. After a block of compounds are screened, the mutant collection is moved on to test the next compound block.

The advantage of this strategy is that the effect of a compound on all the selected mutant strains can be obtained within a relatively short time. This provides compound-focused information for prioritization of compounds in follow-up studies. Since this strategy has only one mutant instead of many mutants on a plate, cross comtamination between different strains and the testing of different mutants at different temperatures (or with other changes in assay conditions) are no longer problems. Moreover, this strategy retains the same compound arrangement in all compound plates, thus saving time, effort and compounds as compared to screening one compound

against many mutants on one plate, for compound focused analysis.

Example 4: Prototype Screening Protocol

S. aureus bacterial cells from pre-prepared frozen stocks are diluted into Mueller-Hinton (MH) broth to an OD600 of about 0.01 and grown at 30°C till OD600=0.5. Cells are diluted 1,000-fold into MH broth and 50 µl is added to each well of 96-well plates to which 40 µl of MH broth and 10 µl of test compound (varying concentrations) are added. No-compound wells with or without cells are included as controls. The total volume in each well is 100 µl. The plates are incubated at an appropriate screening temperature for 20 hr and OD600 are read. The effect of each compound on a mutant is measured against the growth control and % of inhibition is calculated. Wild type cells are screened at the same conditions. The % of inhibition of a compound on a mutant and that on the wild type cell are compared, and compounds that show higher inhibition on the mutant than on the wild type are identified.

3. Screening Method Refinement

Certain testing parameters for the genetic potentiation screening methods can significantly affect the identification of growth inhibitors, and thus can be manipulated to optimize screening efficiency and/or reliabilty. Notable among these factors are variable thermosensitivity of different ts mutants, increasing hypersensititivy with increasing temperature, and

"apparent" increase in hypersensitivity with increasing compound concentration.

a. Variable Thermosensitivity

To use *S. aureus* ts mutants in genetic potentiation screening, the growth of these mutants at different temperatures were measured to determine screening temperatures for each of these mutants. The results showed that different ts mutants have quite different maximum growth temperatures (MGT). The MGTs of some mutants are as high as 39°C, while those of others are 37°C, 35°C, 32°C or even 30°C (Fig. 6). Furthermore, different mutants that have mutations in the same gene may have quite different MGTs, as illustrated in Fig.7 for several *polC* mutants. Thus, different screening temperatures should be chosen for these mutants in order to accommodate the different growth preferences.

b. Raising screening temperature makes ts mutants more sensitive to certain compounds.

To demonstrate that the ts mutants are more sensitive to potential inhibitors at elevated temperature, the effect of different temperatures on the sensitivity of several ts mutants to a subset of compounds was examined. Figure 8 shows the inhibitory effect of 30 compounds on mutant NT99 at 3 different temperatures, 32°C, 35°C, and 37°C. Most of these compounds showed increasing inhibitory effect as temperature increased from 32° to 35°C then to 37°C. Consequently, more hits were identified at 37°C (Fig. 9). In fact, all the hits identified at 32°C and 35°C were

included in the 37°C hits. On the other hand, little difference was observed when the compounds were tested on wild type cells at the same three different temperatures (data not shown).

The temperature effect as mentioned above can be used to control hit rates in the screening. Higher screening temperature can be used to produce more hits for mutants that have low hit rates. Similarly, if a mutant shows a very high hit rate, the number of hits can be reduced by using lower screening temperatures to facilitate hit prioritization.

c. Increasing compound concentrations affect apparent hypersensitivity

The concentration of compounds used in the screening is an important parameter in determining the hit rates and the amount of follow-up studies. The concentration of 10 μg/ml has been used in piloting screening studies. To examine whether screening at lower concentrations can identify a similar set of hits, 41 compounds previously scored as hits were screened agaist their corresponding hypersensitive mutants at lower concentrations. Results in Fig. 10 showed that the number of compounds to which the target mutants were still hypersensitive (≥80% inhibition) decreased as the screening concentrations decreased. At 2μg/ml, only 20 out of 41 hit compounds were able to be identified as hits that inhibit the mutants by ≥80%, and at 1 μg/ml only 11, or 27%, of the compounds still fell into this catagory. These data suggest

that screening at concentrations <2 μg/ml may miss at least half of the hits that would be identified at 10 μg/ml. On the other hand, screening at concentrations higher than 10μg/ml may result in large number of low quality hits and create too much work in hit confirmation and follow-up studies. At 10 µg/ml, a hit may appear as a growth inhibitor for both the mutant and wild type strains. This should not be a major problem since lower concentrations of the compound can be tested in the follow-up studies to differentiate its effect on the mutant and the wild type.

4. Evaluation of uncharacterized known growth inhibitors

In addition to testing known inhibitors of cellular pathways, uncharacterized growth inhibitors identified in other whole-cell screens were also evaluated using temperature sensitive mutants. These growth inhibitors had uncharacterized targets of action. These compounds were previously shown to cause some growth inhibition of the *S. aureus* strain 8325-4 at 5 mg/ml. The compounds were subsequently tested using a range of concentrations against a collection of *S. aureus* ts mutants (all derived from *S.* aureus 8325-4), to determine the MIC values, relative to wild type. Figure 12 summarizes the data generated using 52 *S. aureus* ts mutants and 65 growth inhibitor compounds (47 compounds not shown). The table reports the fold-increase in susceptibility of the ts mutants compared with the wild-type parent strain; values

within two-fold of wildtype have been left blank in the table for ease of identifying the significant hypersensitive values.

The effects of the 65 test compounds on the ts mutants were mostly selective: for most compounds, a limited number of mutants were hypersensitive. Approximately one-third of all compounds showed identical inhibition of mutant and wild type strains (*i.e.*, no mutants were hypersensitive to these compounds). Two compounds in Figure 12 showed strong inhibitory effects on about 50% of the mutants tested (compounds 00-2002 and 00-0167). Two additional compounds showed identical inhibition profiles (compounds 30-0014 and 20-0348, Figure 12). A preliminary analysis of these profiles is provided below.

The genetic basis of the hypersensitivity has been substantiated by two criteria. First, one compound (10-0797) strongly inhibited two mutants (NT52 and NT69) that both affect the same gene. Secondly, complementation of the temperature sensitive phenotype of these mutants resulted in loss of hypersensitivity.

Furthermore, the two compounds that had identical inhibition profiles (30-0014 and 20-0348) have very similar structures (Figure 11). Thus, the hypersensitivity profile provides a pattern that allows recognition of compounds with similar targets of action, even when the target may be poorly defined. The strong similarity in the structures of these compounds makes their common target of action likely. Based on the mutants that were inhibited (*secA* , *dnaG*, and

3 uncharacterized mutants) the target of action of these compounds is not yet defined.

It is preferable to perform a screen of the uncharacterized inhibitors against a larger number of ts mutants. This screen employs preset compound concentrations and obtains the mutant inhibition profile for each compound. Computing the difference in the relative growth of parent and mutant strains in the presence of compounds provides a compound profile similar to that obtained by the MIC determinations of the first screen above.

A wide range of test compounds can be screened. Test compounds that are inhibitory for the wild type parent strain at the pre-selected concentration in the first screening run are retested at a lower concentration to generate an inhibition profile. Data analysis from the screens described above showed that a significant growth reduction of mutant strains compared to the parent strain in the presence of the test compounds is a reasonable indicator of selective compound activity.

Further, compounds for testing can include compounds that show no growth inhibition of the wild type strain. The hypersensitivity of the mutant strains provides the ability to identify compounds that target an essential cellular function, but which lack sufficient potency to inhibit the growth of the wild type strain. Such compounds are modified using medicinal chemistry to produce analogs with increased potency.

The grid shown in Figure 13 represents different mutant inhibition profiles anticipated from screening of growth inhibitors, where "x" denotes inhibition of a particular mutant by a particular compound at concentrations much lower than for wildtype.

This grid shows compounds that cause growth inhibition of more than one mutant (compounds A,C,D,E), compounds that inhibit just one mutant (compounds B,F) and one compound that inhibits no mutants (compound G). In addition, this profile identifies mutants inhibited by no compound (mutant 8), a single compound (mutants 1,6,7), and several compounds (mutants 2,3,4,5). In the preliminary screens described above, compounds were identified that fit some of these anticipated inhibition profiles (see Fig. 14).

In the preliminary screen, compounds that inhibit the growth of the wild type strain were diluted to a point where growth inhibition of wild type no longer occurred. In this situation, only mutants that are hypersensitive to a particular compound will fail to grow. Thus, even compounds considered "generally toxic" should show some specificity of action, when assayed with the hypersensitive mutant strains.

In the simplest interpretation, compounds that cause growth inhibition inhibit the function of one essential macromolecule. Some compounds may specifically inhibit more than one target macromolecule. However, since one of the targets will be most sensitive to inhibition, one target can be considered the primary target. Thus, a

one-to-one correspondence between inhibitors and targets can be established. However, both the data, and less simplistic reasoning provide exceptions to the simple one-to-one relationship between targets and inhibitors. Further analysis and understanding of the complicating effects is necessary to make full use of the data. Some of the complicating effects are discussed below.

a. Compounds that affect many mutants. Certain compounds, such as detergents that target membrane integrity, or DNA intercalators, will have "general", rather than specific targets. These "general targets" are not the product of a single gene product, but rather are created by the action of many gene products. Thus, in analyzing hypersensitivity profiles, compounds that affect many mutants may indicate action on a "general target". The profiles of known membrane active agents, and intercalators will provide information to recognize uncharacterized compounds with similar effects.

Compounds that cause growth inhibition of more than one mutant may also arise when the affected mutants are metabolically related. These mutants may affect the same gene, or the same biochemical pathway. For example, mutants defective in one of many cell wall biosynthetic steps may show hypersensitivity to compounds that inhibit any of these steps. Evidence for this type of effect was observed in the hypersensitivity patterns of known inhibitors (see Figure 2). This concept can be broadened to include effects caused by the "metabolic web", in which far-reaching consequences

may arise through characterized and uncharacterized interrelationships between gene products and their functions.

Overall, the hit rate was high when we considered all compounds that were more active on mutants than on the parent strain. The histogram in Figure 14 shows the hit rate for compounds that affected one, two, three, or more than three mutants in our prototype screen. The large number of compounds that affected more than three different mutants was at least partly explained by the greater potency of this group of compounds. Figure 15 illustrates the potency of some of the hits found in the screen as evaluated by the MIC obtained for the parent strain *S. aureus* 8325-4.

In the prototype screen, compounds affecting more than 3 mutants were generally more potent but some may also be considered broadly toxic. The columns identified by an asterisk in Figure 15 represent 3 out of 4 compounds that were also shown to be inhibitors of *Salmonella typhimurium* in another whole cell screen. Consequently, only the most hypersusceptible strain of a group of mutants affected by the same compound should be considered as the primary target. However, the entire mutant inhibition profile of a specific compound is very useful and should be considered as its actual fingerprint in pattern recognition analysis.

b. <u>Compounds that affect few (or no) mutants</u>. Since all compounds assayed in the preliminary screen inhibit the growth of the wild type strain to some degree (initial basis of pre-selection), such compounds

indicate that the mutant population is not sufficiently rich to provide a strain with a corresponding hypersensitive target.

c. Mutants affected by many compounds.

Another complication of the simple one-to-one compound/target relationship will arise because of mutants that are inhibited by many different compounds. The relative number of compounds (% hits) that inhibited the growth of each mutant in the *S. aureus* pilot is shown in Figure 16. Several mutants were affected by many compounds. Several distinct causes of this are apparent. First, some mutants may have defects in the membrane/barrier that cause hyperpermeability to many different compounds. Such mutants will have higher intracellular concentrations of many compounds, which will inhibit metabolically unrelated targets. Other mutants may have defects that have far-reaching consequences, because their gene products sit at critical points in the metabolic web. Still other mutants may have specific alleles that are highly crippled at the assay temperature. For these mutants, the metabolic web consequences are large because the specific allele has created a highly hypersensitive strain.

d. Mutants affected by few or no compounds.

For the mutants that were hypersusceptible to fewer compounds, it is possible that their mutations affect a limited metabolic web, that mutations provide a true specificity that was yet not revealed by any compound, or that these mutants have nearly full activity at the assay

temperature. This analysis stresses the importance of strain validation as indicated above.

In interpreting these patterns, the number of mutants screened and the total number of targets are also important variables. These numbers provide a simple probabilistic estimate of the fraction of the compounds that should have a one-to-one correspondence with a mutant target in the sample that was screened.

6. <u>Prioritization of Hits and Downstream Development</u>

The early steps in a multi-channel genetic potentiation screen include the following:

- Pre-selection of mutant strains for screening
- Pre-selection of desired test compounds based on structural features, biological activity, etc. (optional)
- Testing of the chosen compounds at a pre-determined concentration, preferably in the range 1-10 μg/ml.
- Analysis of inhibitory profiles of compounds against the mutant population and selection of interesting hits
- Confirmation of the selective inhibitory activity of the interesting hits against specific mutants
- Secondary evaluation of prioritized hits.

Genetic potentiation assays provide a rapid method to implement a large number of screens for inhibitors of a

large number of targets. This screening format will test the capacity of rapid high-throughput screening. The capability to screen large numbers of compounds should generate a large number of "hits" from this screening. Limitations in downstream development through medicinal chemistry, pharmacology and clinical development will necessitate the prioritization of the hits. When large numbers of hits are available, each with reasonable *in vitro* activity, prioritization of hits can proceed based on different criteria. Some of the criteria for hit characterization include:

- chemical novelty
- chemical complexity, modifiability
- pharmacological profile
- toxicity profile
- target desirability, ubiquity, selectivity

Secondary tests will be required not only for the initial evaluation of hits, but also to support medicinal chemistry efforts. While the initial genetic potentiation tests will be sufficient to identify and confirm hits, selection of hits for further development will necessitate establishment of the specific target of action. Equipped with the gene clones, selection of resistant alleles provides early evidence for the specific target. Subsequent efforts to establish a biochemical assay for rapid, specific and sensitive tests of derivative compounds will be aided by

the over-expression and purification of the target protein, sequence analysis of the ORF to provide early insight into novel target function, as well as a variety of physiological and biochemical tests comparing the mutant and wild type strain to confirm the novel target function, and aid in the establishment of biochemical assays for the targets.

7. <u>Identification of Specific Inhibitors of Gene Having Unknown Function</u>

In a piloting screening study, a number of compounds were identified as inhibitors for mutants with mutations located in open reading frames whose functions are not known. Some of the open reading frames have been previously identified in other bacteria while others show little homology to the current Genbank sequence collection. An example is mutant NT94, whose complementing clones contain an open reading frame that is homologous to a spoVB-like gene in *B. subtilis*. While the function of the gene is not clear in either *B. subtilis* or *S. aureus*, NT94 is hypersensitive to many compounds tested, as illustrated in Table 2 below.

Table 2

Hit Rates in Genetic Potentiation Screen

Number of mutants n, on which cmpds active		Confirmed Hits	
		39 mutants	NT94
n = 1 or 2	Average hit rate	0.03%	1.06%
	Hit rate range among mutants	0 - 0.31%	
n => 3	Average hit rate	0.17%	1.39%

Table 2

Hit Rates in Genetic Potentiation Screen

	Hit rate range among mutants	0 - 0.72%	

In fact, NT94 had the highest hit rate among the 40 mutant strains tested. Among the NT94 hits, 4 compounds share similar chemical structures (Figs. 19A-D) The MICs of these compounds on NT94 are 0.25-2 μg/ml, which are 16-256 fold lower than those on the wild type cells (32-64 μg/ml). The similarity in the compound structures suggests a common and specific mechanism of the inhibitory effect on NT94.

Furthermore, the hypersensitivity to these compounds can be abolished by introducing 2 or more copies of the wild type gene into NT94. A correlation between the copy number of the wild type gene and the tolerance to the compounds has been observed. Cells with 2 copies of the wild type gene are slightly more resistant (2-fold increase in MIC) to MC-207,301 and MC-207,330 than the wild type cells which has one gene copy; cells carrying complementing plasmids (about 20-50 copies per cell) are much more resistant (8-16 fold increase in MIC). Such a gene dosage effect further suggests that either the gene product itself or its closely related functions of the open reading frame affected in NT94 is the target of the hit compounds.

8. Multi-Channel Screen Advantages

As depicted by the *S. aureus* example shown above, multi-channel screen design rapidly leads to the identification of hits and provide some of the necessary

specificity information to prioritize compounds for further evaluation. Figure 17 illustrates the advantages of a genetic potentiation approach as the basis of a screen design.

Overall, an approach using whole-cell genetic potentiation of ts mutants includes the selectivity of the biochemical screens (it is target-specific, or at least pathway-specific) and it is more sensitive than traditional screens looking for growth inhibitors due to the hypersensitive nature of the mutants. This genetic potentiation approach also provides a rapid gene-to-screen technology and identifies hits even before the genes or biochemical targets are fully characterized.

9. Alternatives to Ts Hypersensitivity Screening

There are a number of additional strategies that can be undertaken to devise target-based whole cell screens, as well as binding or biochemical type screens. In order to implement these strategies, knowledge of the existence of the gene, the DNA sequence of the gene, the hypersensitivity phenotype profile, and the conditional mutant alleles will provide significant information and reagents. Alternative strategies are based on:

- over- and under-expression of the target gene
- dominant mutant alleles
- hypersensitive mutant alleles

a. Over- and Under-expression of Target Genes. There are numerous examples of over-expression phenotypes that range from those caused by 2-fold increases in gene dosage (Anderson and Roth, 1977, *Ann. Rev. Microbiol.* 31:473-505; Stark and Wahl, 1984, *Ann. Rev. Biochem.* 53:447-491) to multi-fold increases in dosage which can be either chromosomal-encoded (Normark et al., 1977, *J. Bacteriol.* 132:912-922), or plasmid-encoded (Tokunaga et al., 1983, *J. Biol. Chem.* 258:12102-12105). The phenotypes observed can be analog resistance (positive selection for multiple copies, negative selection for inhibition phenotype) or growth defects (negative selection for multiple copies, but positive selection for inhibition phenotype).

Over-expression can be achieved most readily by artificial promoter control. Such screens can be undertaken in *E. coli* where the breadth of controllable promoters is high. However, this method loses the advantage gained by whole cell screening, that of assurance that the compound enters the pathogen of interest. Establishing controllable promoters in *S. aureus* will provide a tool for screening not only in *S. aureus* but most likely in other Gram-positive organisms. An example of such a controllable promoter is shown by controlled expression of the *agr* P3 promoter in the *in vivo* switch construction.

b. Dominant alleles. Dominant alleles can provide a rich source of screening capabilities. Dominant alleles in essential genes will prevent growth unless

conditions are established in which the alleles are non-functional or non-expressed. Methods for controlled expression (primarily transcriptional control) will provide the opportunity to identify dominant mutant alleles that prevent cell growth under conditions of gene product expression.

Equally useful will be mutant alleles that are dominant, but conditionally functional. A single mutation may provide both the dominant and conditional-growth phenotype. However, utilizing the existing collection of temperature sensitive alleles, mutagenesis with subsequent selection for a dominant allele may provide more mutational opportunities for obtaining the necessary dominant conditional alleles. There is precedent for such additive effects of mutations on the protein phenotype (T. Alber, 1989, *Ann. rev. Biochem.* 58:765-798) as well as evidence to suggest that heat-sensitive mutations, which generally affect internal residues (Hecht et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:2676-2680), will occur at different locations in the protein different than dominant mutations, one type of which will affect protein-protein interactions, which are more likely on the protein surface.

The use of dominant conditional double mutants may have an additional advantage, since the hypersensitivity phenotypes may remain the same in the double mutant as in the single conditional mutant allele. In this case, a merodiploid carrying two copies of the target gene - one wild type, and one carrying the dominant conditional doubly

mutant gene - would provide a sophisticated screening strain (see Figure 18). The screen would rely on the hypersensitivity of the dominant protein to inhibitor compounds. Under conditions of the dominant protein's function, cells will not grow, while inhibition of the dominant protein will allow cell growth. The temperature sensitive allele provides a basis for hypersensitivity of the dominant protein, relative to the wild type protein.

c. Hypersensitive mutant alleles - Additional mutants that display more pronounced hypersensitivities than the original conditional lethal mutants can be sought. Selection or screening procedures are based on the initial secondary phenotype profiles. These new highly hypersensitive alleles need not have a conditional growth defect other than that observed in the presence of the toxic agent or inhibitor. Such highly hypersensitive alleles provide strong target specificity, and high sensitivity to weak inhibitors. Such hypersensitive alleles can readily be adapted for screens with natural products, and with synthetic or combinatorial libraries of compounds in traditional screen formats.

d. Compound Binding and Molecular Based Assays and Screens

As indicated above, knowledge and possession of a sequence encoding an essential gene also provides knowledge and possession of the encoded product. The sequence of the gene product is provided due to the known genetic code. In addition, possession of a nucleic acid sequence encoding a

polypeptide provides the polypeptide, since the polypeptide can be readily produced by routine methods by expressing the corresponding coding sequence in any of a variety of expression systems suitable for expressing procaryotic genes, and isolating the resulting product. The identity of the isolated polypeptide can be confirmed by routine amino acid sequencing methods.

Alternatively, once the identity of a polypeptide is known, and an assay for the presence of the polypeptide is determined, the polypeptide can generally be isolated from natural sources, without the necessity for a recombinant coding sequence. Such assays include those based on antibody binding, enzymatic activity, and competitive binding of substrate analogs or other compounds. Consequently, this invention provides purified, enriched, or isolated products of the identified essential genes, which may be produced from recombinant coding sequences or by purification from cells naturally expressing the gene.

For use of binding assays in screening for compounds active on a specific polypeptide, it is generally preferred that the binding be at a substrate binding site, or at a binding site for an allosteric modulator, or at another site which alters the relevant biological activity of the molecule. However, simple detection of binding is often useful as a preliminary indicator of an active compound; the initial indication should then be confirmed by other verification methods.

Binding assays can be provided in a variety of different formats. These can include, for example, formats which involve direct determination of the amount of bound molecule, either while bound or after release; formats involving indirect detection of binding, such as by determination of a change in a relevant activity, and formats which involve competitive binding. In addition, one or more components of the assay may be immobilized to a support, though in other assays, the assays are performed in solution. Further, often binding assays can be performed using only a portion of a polypeptide which includes the relevant binding site. Such fragments can be constructed, for example, by expressing a gene fragment which includes the sequence coding for a particular polypeptide fragment and isolating the polypeptide fragment, though other methods known to those skilled in the art can also be used. Thus, essential genes identified herein provide polypeptides which can be utilized in such binding assays. Those skilled in the art can readily determine the suitable polypeptides, appropriate binding conditions, and appropriate detection methods.

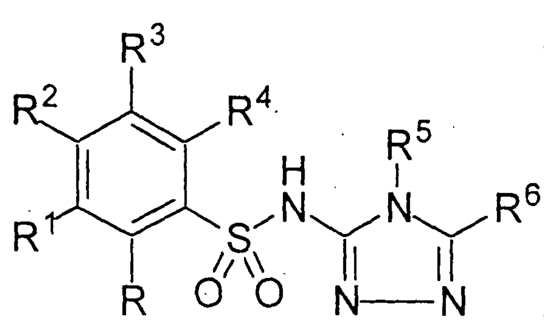
Provision of a purified, enriched, or isolated polypeptide product of an essential gene can also allow use of a molecular based (*i.e.*, biochemical) method for screening or for assays of the amount of the polypeptide or activity present in a sample. Once the biological activities of such a polypeptide are identified, one or more of those activities can form the basis of an assay for the

presence of active molecules of that polypeptide. Such assays can be used in a variety of ways, for example, in screens to identify compounds which alter the level of activity of the polypeptide, in assays to evaluate the sensitivity of the polypeptide to a particular compound, and in assays to quantify the concentration of the polypeptide in a sample.

10. Antibacterial Compounds Identified by Hypersensitive Mutant Screening

Using the genetic potentiation screening methods described above, a number of compounds have been identified which inhibit growth of S. aureus cell. These compounds were identified as having activity on the NT94 mutant described above, and so illustrate the effectiveness of the claimed screening methods. These results further illustrate that the genes identified by the temperature sensitive mutants are effective targets for antibacterial agents. The identified compounds have related structures, as shown in Figs. 19A-D

These compounds can be generally described by the structure shown below:



in which

R, R^1, R^2, and R^3 are independently H, alkyl (C_1-C_5),or halogen;

R^4 is H, alkyl (C_1-C_5), halogen, SH, or S-alkyl (C_1-C_3);

R^5 is H, alkyl (C^1-C^5), or aryl (C_6-C_{10});

R^6 is CH2NH2, alkyl (C1-C4), 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, or aryl (C_6-C_{10});

or

R^5 and R^6 together are $-C(R^7)=C(R^8)-C(R^9)=C(R^{10})-$, $-N=C(R^8)-C(R^9)=C(R^{10})-$, $-C(R^7)=N-C(R^9)=C(R^{10})-$, $-C(R^7)=C(R^8)-N=C(R^{10})-$, or $-C(R^7)=C(R^8)-C(R^9)=N-$;

in which

R^7, R^8, R^9, and R^{10} are independently H, alkyl (C_1-C_5), halogen, fluoroalkyl (C_1-C_5);

or

R^7 and R^8 together are -CH=CH-CH=CH-.

Thus, the invention includes antibacterial compositions containing the described compounds, and the use of such compositions in methods for inhibiting the growth of bacteria and methods for treating a bacterial infection in an animal.

<u>V. Description of Compound Screening Sources and Sub-structure Search Method</u>

The methods of this invention are suitable and useful for screening a variety of sources for possible activity as inhibitors. For example, compound libraries can be screened, such as natural product libraries,

combinatorial libraries, or other small molecule libraries. In addition, compounds from commercial sources can be tested, this testing is particularly appropriate for commercially available analogs of identified inhibitors of particular bacterial genes.

Compounds with identified structures from commercial sources can be efficiently screened for activity against a particular target by first restricting the compounds to be screened to those with preferred structural characteristics. As an example, compounds with structural characteristics causing high gross toxicity can be excluded. Similarly, once a number of inhibitors of a specific target have been found, a sub-library may be generated consisting of compounds which have structural features in common with the identified inhibitors. In order to expedite this effort, the ISIS computer program (MDL Information Systems, Inc.) is suitable to perform a 2D-substructure search of the Available Chemicals Directory database (MDL Information Systems, Inc.). This database contains structural and ordering information on approximately 175,000 commercially available chemical compounds. Other publicly accessible chemical databases may similarly be used.

VI. *In vivo* modeling: Gross Toxicity

Gross acute toxicity of an identified inhibitor of a specific gene target may be assessed in a mouse model. The inhibitor is administered at a range of doses, including high doses, (typically 0 - 100 mg/kg, but preferably to at least 100 times the expected therapeutic dose)

subcutaneously or orally, as appropriate, to healthy mice. The mice are observed for 3-10 days. In the same way, a combination of such an inhibitor with any additional therapeutic components is tested for possible acute toxicity.

VII. Pharmaceutical Compositions and Modes of Administration

The particular compound that is an antibacterial agent can be administered to a patient either by itself, or in combination with another antibacterial agent, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). A combination of an inhibitor of a particular gene with another antibacterial agent can be of at least two different types. In one, a quantity of an inhibitor is combined with a quantity of the other antibacterial agent in a mixture, *e.g.*, in a solution or powder mixture. In such mixtures, the relative quantities of the inhibitor and the other antibacterial agent may be varied as appropriate for the specific combination and expected treatment. In a second type of combination an inhibitor and another antibacterial agent can be covalently linked in such manner that the linked molecule can be cleaved within the cell. However, the term "in combination" can also refer to other possibilities, including serial administration of an inhibitor and another antibacterial agent. In addition, an inhibitor and/or another antibacterial agent may be administered in pro-drug forms, *i.e.* the compound is administered in a form which is

modified within the cell to produce the functional form. In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of an agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound(s) that results in amelioration of symptoms or a prolongation of survival in a patient, and may include elimination of a microbial infection.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. It is preferable that the therapeutic serum concentration of an efflux pump inhibitor should be in the range of 0.1-100 μg/ml.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated

initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, *e.g.*, Fingl et al., in THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific infection being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal,

transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art, into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers including excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension,

such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

VIII. Use of Gene Sequences as Probes and Primers

In addition to the use of the growth conditional mutant strains as described above, DNA sequences derived from the identified genes are also useful as probes to identify the presence of bacteria having the particular gene or, under suitable conditions, a homologous gene. Similarly, such probes are useful as reagents to identify DNA chains which contain a sequence corresponding to the probe, such as for identifying clones having a recombinant DNA insert (such as in a plasmid). For identifying the presence of a particular DNA sequence or bacterium having that sequence it is preferable that a probe is used which will uniquely hybridize with that sequence. This can be accomplished, for example, by selecting probe sequences from variable regions, using hybridization conditions of suitably high stringency, and using a sufficiently long probe (but

still short enough for convenient preparation and manipulation. Preferably, such probes are greater than 10 nucleotides in length, and more preferably greater than 15 nucleotides in length. In some cases, it is preferable that a probe be greater than 25 nucleotides in length. Those skilled in the art understand how to select the length and sequence of such probes to achieve specific hybridization. In addition, probes based on the specific genes and sequences identified herein can be used to identify the presence of homologous sequences (from homologous genes). For such purposes it is preferable to select probe sequences from portions of the gene which are not highly variable between homologous genes. In addition, the stringency of the hybridization conditions can be reduced to allow a low level of base mismatch.

As mentioned above, similar sequences are also useful as primers for PCR. Such primers are useful as reagents to amplify the number of copies of one of the identified genes or of a homologous gene. As with probes, it is preferable that the primers specifically hybridize with the corresponding sequence associated with one of the genes corresponding to SEQ ID NO. 1-105. Those skilled in the art understand how to select and utilize such primers.

The embodiments herein described are not meant to be limiting to the invention. Those of skill in the art will appreciate the invention may be practiced by using any of the specified genes or homologous genes, for uses and by

methods other than those specifically discussed, all within the breadth of the claims.

Other embodiments are within the following claims.